STUDIES ON THE MYCETOMAL MICROORGANISM ASSOCIATED WITH CLOVIA PUNCTA WALKER.

DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT FOR THE DEGREE OF

Master of Philosophy

IN

ZOOLOGY

BY

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APRIL, 1981

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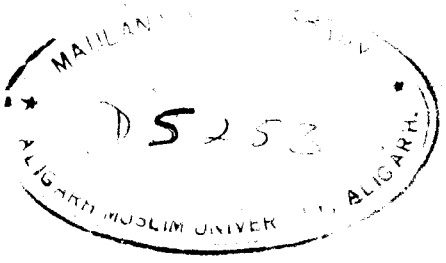
This is to certify that the dissertation for M. Phil. in Zoology has been completed by Mr. Munney Miyan under my supervision. It is original in nature and I have permitted the candidate to submit it for the award of M. Phil. degree in partial fulfilment of M. Phil. requirements in Zoology.

(Abeer M. Khan)



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ABSTRACT

The jassid, Clovia punata Walker is an important agricultural pest in northern India and was therefore selected to study if it had any relationship with the mycetomes. The species did showed the presence of mycetomes which were elongated, yellowish in colour and are situated between sixth to eight abdominal segments. The bacterium isolated from the mycetomes was grown on different artificial media in vitro and on the basis of its morphological, cultural studies and biochemical reactions, it was identified as Klebsiella sp.

The sensitivity test by disc method with different antibiotics showed that the Klebsiella sp. was highly sensitive to althrocin and chloromycetin but it was least susceptible to streptomycin.

Various concentrations (0.1%, 0.2% and 0.3%) of different chemicals (sugars, vitamins, antibiotics and insecticides) were used during the present investigations. Observations were started from 0.0 hr to 144.0 hrs at intervals of 12 hrs except for the second observation which was taken at 6 hrs. after incubation in all the tests conducted by the author. The growth of bacterium was expressed in terms of percentage transmittance with

turbidity methods with the help of a colorimeter. Green filter was used throughout the experiments. All observations were illustrated graphically and their numerical values were analysed statistically.

The chemicals used in this bio-assay can be classified into two categories; (i) the growth promoting which includes sugars and vitamins, (ii) the growth inhibiting including antibiotics and insecticides. Nearly all sugars were found supporting growth of the bacterium. Most easily fermented sugars were maltose and lactose followed by starch and sucrose while others were less easily fermented. In general vitamins accelerated growth especfically vitamin B_{12} and B complex were found most effective. It was interesting to find that some vitamins (vitamin B_{12}, E, K and C) enhanced the lag phase of this bacterium.

Highly effective antibiotics were althrocin and chloromycetin followed by terramycin, gentiayn, penicillin and streptomycin. Althrocin and chloromycetin greatly inhibited the growth while others were moderate in their action. Penicillin and streptomycin which were found to be least effective. Highest bactericidal action was shown by DDT and BHC followed by sodium arsenate and zinc sulphate, calcium arsenate and furadon showed little bactericidal action.

I N T R O D U C T I O N

INTRODUCTION

The association of micro-organism with the insects has been established since a long time Blochmann (1886), Buckner (1912), Glaser (1920) and Steinhaus (1949 and 1940,a) These micro-organisms existed both internally and externally. The internal microflora have biological relationship with insects and affects each of these forms of life upon the other. These organisms are adaptive to physiological processes involved in their association and affect the respective relationship and development. Internally, the micro organisms may be associated extracellularly in the alimentary tract, cecae, pouches, diverticula and in the Haemocoel, or they may be intracellularly in the epithelial lining of the alimentary tract, malpighian tubes, salivary glands, or they may be in the specialized cells, The mycetocytes in organs, the mycetomes. This association may be symbiotic, parasitic, mutualistic and commensal type or exist only in casual or accidental association with the insect, the fortuitous form.

The mycetomal microorganisms harbouring tissues, had drawn the attention of early workers like Buchner (1912)(1923), Glaser (1924 a), Mahdi Hasan (1939, 1941) and Steinhaus (1941a) These workers extensively studied the morphology, histology and tried to culture them (*in vitro*) on artificial media and

established the symbiotic nature of the association in aphids, Cimex lectularis and Rhizopertha dominica. In Macrosiphum rosae it also provides nutrition supplying good materials to the growing embryo.

Recently Brooks & Richards (1956), Brooks (1963), Gabrani (1970) and Khan (1974) have isolated the symbiotes from the mycetomes Periplaneta americana, Sitophilus oriza and Dysdercus cingulatus respectively and successfully cultured them on artificial media. Steinhaus (1949) isolated the symbiotics from the mycetomes and mycetocytes, while claiming similar views, he pointed out that the symbionts could exist as a free living organism. Their possible mode of existence represent the independent relationship between micro organisms and host insects. Further, in other aspects of utilizing the microorganisms as disease producing agents in the host species. The entomogenous bacteria were successfully utilized, particularly the Bacillus sp. which form the most promising alternative and can be of more use in the integrated pest management programme, Majumder et al., 1955 & 56, Heimpel (1967) and Dutky (1959). Bacillus thuringiensis is commonly used and recommended for the control of large number of pests. It is particularly highly effective against large number of lepidopterous foliage feeders, Majumder et al. (1955,56) and this aspect is further strengthen by Angus (1954 & 1956) on various insects.

Microorganisms differ to a great extent in the range of organic compounds that can be utilized as a source of carbon and energy. Some bacteria are conspicuous, such as species of genus Pseudomonas and Bacillus coli which can be utilised over a hundred organic compounds (Sugars, acids and alcohols) as the main source of carbon and energy whereas many bacteria are much more specific in their requirements. Singh & Pant (1955) and Khan & Zaidi (1976).

The growth of micro-organisms are dependent on an adequate supply of suitable metabolites, the nutrients. The specific requirements vary according to the natural environmental adaptation of different species. Some bacteria are able to grow under wide range of conditions, but others especially the more strictly parasite such as gonococcus, are very exacting and restrictive in their requirements. Some species do differ widely in their requirements for amino acids, nucleotides and growth factors (Cornner & Hensen, 1967). Therefore, in their synthetic capacity, some especially among the non-parasites species, e.g., Klebsiella aerogenes have comprehensively synthetic abilities and are therefore, non exacting nutritionally. They are able to synthesize all their structural unit from the carbon and energy sources and inorganic salts.

The main elements required for growth are carbon, hydrogen, oxygen and nitrogen; with sulphur and phosphorus required in smaller amounts and other elements such as sodium, potassium, magnesium, iron and manganese in considerably small quantities. Since hydrogen and oxygen can be obtained from water, the other requirements are completed by other metabolites. Carbon and energy is obtained from the organic compounds such as carbo-hydrates, aminoacids, lipids, nucleotides and coenzymes. The main nitrogen requirement is accomplished by ammonium salts. The other sources which can be utilised by bacteria, are environmental nitrogen; deamination of amino acids and direct nitrogen fixation from atmosphere. The organisms require a supply of inorganic salts for growth, particularly the anions phosphate and sulphate and the cations, sodium, potassium, magnesium, iron and manganese.(Steinhaus & Birkeland, 1940 a)

The growth of these bacteria is regulated internally and externally both. Internally during the biosynthesis in vitro, the organisms produce toxins, acids and gases which are anti bacterial properties, that is why the fluctuation in the community occurs. (Angus, 1954 & 56; Hempel, 1967) Externally bacterial ecology is affected by temperature, moisture, light radiations and osmotic pressures.

Since the mycetomal bacteria are closely associated with biology of the hosts, their activities can be affected by the introduction of chemicals (sugars, vitamins, antibiotics and insecticides). This idea led the entomologist to eliminate (total or partial) the bacteria from the hosts and affecting the biologies of host species in maintaining the pest population. Koch (1936a) was the first worker who tried to kill symbiotic bacteria of fat body by introducing triflavin in the body cavity of Periplaneta americana. Breus and Dunn (1945) successfully tried to inhibit the mycetomal bacteria with sulphanilamide, sulphathiozole, sulphadiozin, sulphapyridine and sulphanilate. So far extensive work has been done regarding the elimination of symbiotic bacteria by various antibiotics, such as Penicillin, chloromycetin, aureomycin, oxytetracycline, tetracycline, streptomycin and hostamycin. These antibiotics were applied though feed, drinking water or in the form of injections.

The bacteria have varying degree of sensitivity to different chemicals (both insecticides and chemicals). Chlorotetracycline and aureomycin are absolutely effective antibiotics for Blatta germanica (Brook, 1954), B. Orientalis (Frank, 1954, 56), Rhizopertha dominica and Oryzaephilus surinamensis (Huger, 1956).

The symbiotic bacteria have great importance in nutritional requirement of the host species. They maintain their metabolic balance, produce vitamins and also synthesize proteins from nitrogenous metabolic products Wigglesworth (1936) and Baines (1956). In some cases they do assist in digestion of cellulose and other carbohydrates, so that they are thought to be coordinator to some extent between the host and its physiology. The exploitation of the bacteriologic nutritional relationship, is of immense value to the economic entomologists for managing pest population, Lemond & Beamond (1953), Pant & Frenkel (1954) and Khan & Zaidi (1976) have contributed a lot towards the nutrition of micro-organisms in the hosts.

Very recently Khan (1974, 1977) successfully tried to control the insects with the introduction of antibiotics and sulpha drugs. He concluded that these drugs could be used in disturbing the symbiotic balance existing between host and symbionts. In 1976 he again tried certain antibiotics on the artificial media where Klebiella sp. showed high degree of sensitivity, so that these antibiotics could be given (through feed or injected) to eliminate the micro-organisms and disturb the biology of the host species.

Due to lack of literature on the existing relationship of microbes with insects, the information regarding the role of internally harbouring (Mycetomal, intestinal bacteria of salivary gland) bacteria is little. To control the wide variety of pest species, it needs full exploitation of nutritional requirement of bacteria, (mycetomal microorganisms The exploitation will provide us more clues leading to the control of the many noxious species of pests economically and with great safety.

In spite of this, information regarding the role of mycetomal organisms in the life of the insects that are economically important belonging to different orders, is still far from complete. But there are various noxious and agriculturally important insects which have not been dealt with in detail, in this regard. In case role of such organisms is worked out it may prove another suitable method of biological control. In the present investigation, it is, therefore, aimed to isolate, cultivate the bacteria associated with Colyia punata Walk. and to determine the effects of sugars, vitamins, antibiotics and insecticides on the growth of micro organism in vitro which shall be further extended for Ph.D. work.

MATERIALS AND METHODS

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SURVEY OF MYCETOMES

The insects belonging to different orders were collected from experimental fields of Zoology Department and Aligarh Fort, and were dissected under biocular microscope to find out whether the mycetomes were present or absent in these agricultural pests.

ISOLATION AND CULTIVATION OF MYCETOMAL MICRO-ORGANISM

Before isolating the mycetomes, the specimens, C. punata Walk, were etherised; the wings and legs were removed and the remaining portion of the bodies were sterlised in 1:1000 mercuric chloride. Later the insects bodies were rinsed in sterlised normal saline solution. Under sterlised condition in steriscopic microscope, the dissections were performed and mycetomes, after removal from the abdomen, were transferred to culture tubes containing sterlised normal saline. The mycetomes were macerated by means of a sharp needle, transferred to nutrient broth tubes and incubated for 24 hrs at 37°C. Bacteria from all such tubes showing turbidity were plated on different artificial media. The individual colony from the above plates were transferred to nutrient agar slant and incubated at 37°C for 24 hrs. The slants were later stored in refrigerator at 04°C.

ANATOMY, BIOCHEMICAL TESTS AND IDENTIFICATION OF MYCETOMAL MICROORGANISM :

The form, arrangement, size of bacterial cells and its reaction to gram's stain, vegetative growth on different artificial media and biochemical tests were performed. The detailed techniques were same as earlier given by Breed et. al. (1956) and Khan (1977).

ANTIBIOTICS SENSITIVITY TEST BY DISC METHOD :

The antibiotic sensitivity tests on mycetomal bacteria were made by making use of large number of broad spectrum antibiotics. A confluent bacterial growth was first obtained on nutrient agar plates by inoculating the organisms and allowing the period of 8 hrs of incubation by the "Whatmann filter paper discs, soaked in antibiotics and were plated over bacterial carpet. These plates were incubated for a period of 24 hrs at 37°C and zone of inhibition was measured on mm scale. A diameter 15 mm and above were taken as index of inhibition. All the antibiotics, terramycin, Althrocin, Chloromycetin, penicillin, streptomycin and gentcyn were used in 0.1% concentration in sterilised water.

EFFECT OF DIFFERENT SUGARS, VITAMINS, ANTIBIOTICS AND INSECTICIDES ON MYCETOMAL MICROORGANISM :

Various concentrations of these chemicals sucrose, lactose, maltose, dextrose, mannitol, starch, Vitamin-A,

B12, B complex, C, K and E, althrocin, chloromycetin, terramycin, Genticyn, penicillin streptomycin; DDT, BHC, sodium arsenate, zinc sulphate, calcium arsenate and Furadon were made in sterilized double distilled water. The concentration of various chemicals used in the experiment were 0.1%, 0.2% and 0.3%. Each culture tube containing 8.5 ml of nutrient broth was inoculated with 0.5 ml of 24 hrs old bacterial culture and 1.0 ml of various concentration of chemicals. The control test tubes were similarly prepared except the chemical solution which was replaced by equal amount of normal saline. Observations were made at zero, 6 hrs and subsequently at after every 12 hrs of interval upto 144 hrs. Test tubes both experimental and controlled were incubated at 37°C during this period.

Turbidity as a direct function of growth in each test tube was measured in % transmittance with the help of calorimeter upto 144 hrs. All these treatments were replicated thrice and results were analysed statistically.

RESULTS

RESULTS

The dissections were made to confirm the presence of mycetomes in a number of insects belonging to the following orders, Hemiptera, Homoptera, Diptera and Coleoptera. The following insects showed the presence of mycetomes : Clovia punata Walk. Oxyrachia terandus Farb. Dacus cucurbitae Coq. Rhiseperthe dominica Linn. and Sitophilous oriase. In Musca domestica Linn. and Aulacophora faviculis the mycetomes and mycetocytes could not be detected. Detailed study on the mycetomal bacteria associated with C. punata Walk. was made in view of its agricultural importance.

In this insect the mycetome exists in pair. They are elongated yellowish in colour and situated near the 6-8th abdominal segments on the both sides of alimentary canal. Each mycetome is surrounded by the fine tracheal branches. After removal of the fat bodies and tracheal branches, the mycetomes were taken out from the abdomen and were put into normal saline.

The isolated mycetomal organism were transferred into culture tubes containing nutrient broth and these tubes were put for incubation at 37°C for 24 hrs.

After obtaining pure culture the organisms were transferred to different artificial media for cultural studies. The organism was again incubated at 37°C for 24 hrs. The growth of organism in different media showed the following characteristics.

It was rod shaped, measuring 0.3 to 0.5 by 5.0 u occurring singly and in pairs, encapsulated, non motile and gram negative.

Agar culture : Colonies white, slimy convex smooth, glistening and complete.

Agar slant : Slimy, white, some what translucent, raised growth.

Gelatin culture : Dirty white, smooth, opaque, entire and colonies raised.

Gelatin stab : Dirty white, surface growth filiform growth in stab. No liquefaction.

Nutrient broth : Turbid, with thickening or crust.

The biochemical tests show that organism is capable of producing nitrite from nitrates and is also able to ferment glucose, lactose, sucrose, fructose, galactose and mannitol but it was not able to produce H_2S and indole. The organism also gave negative methyl test and positive acetyle methyl carbinol (VP) test. Litmus milk test gave positive acid with no coagulation and there was no haemolysis on blood agar plate. (Table 1). On the basis of above

characteristics the organism is identified as Klebsiella sp.

The smear preparations of the mycetomes show large number of red coloured rod shaped bodies fairly uniform in size and shape, some of them being minute and granular. The disintegrated cytoplasm takes bluish stain. Large number of small as well as large vacuolated spaces are found filled with rod shaped and granular microorganisms. A good number of red coloured giant nuclei is a typical pathological condition which may be due to invasion of the microorganisms.

SENSITIVITY AGAINST ANTIBIOTICS (BY DISC METHOD) :

The sensitivity pattern of various antibiotics were studied by measuring the zone of inhibition. The details of inhibition zone obtained by different antibiotics are shown in Table 2. The sensitivity pattern of various antibiotics as shown by disc method reveals that chloromycetin and althrocin are found most effective and produced highly marked inhibition zone. It is followed by penicillin, gentioyn and terramycin while streptomycin fails to inhibit the growth of Klebsiella sp. in vitro.

EFFECT OF VARIOUS CHEMICALS ON THE GROWTH OF Klebsiella sp. BY TURBIDITY METHOD :

Results are graphically illustrated where the concentrations are plotted against growth index values and the variations of growth curves obtained (Fig. 1-24).

The growth curves have a tendency to rise in low concentration and also with the increase in time period of exposure of bacterium to chemicals. When the concentrations of various chemicals (growth promoting) increase the growth curves decline rapidly. While in other chemicals (growth limiting) the adverse reactions were observed.

EFFECT OF SUGARS :

All sugars are fermented indicating that they are utilized by the bacterium. Most easily fermented sugars are maltose and lactose followed by starch and sucrose, while dextrose and mannitol are slightly less easily fermented

The results indicate that in maltose (Fig. 1) the multiplication of bacteria is most rapid, with heavy turbidity reaching 24%, 24% and 21% transmittance in 0.1%, 0.2% and 0.3% concentrations after 12 hrs (Tables, 3,4 & 5). As the concentration increased the rate of growth increases but when concentration is increased beyond the optimum level, there is a retardation of growth of Klebsiella sp. After 24 hrs. the growth slowly declines.

It is evident from the Fig. 2 that in lactose solution the highest growth is observed after 24 hrs showing 24%, 27% and 25% transmittance in 0.1%,02% and 0.3%

concentrations respectively, Apparently, it is clear from the figure (2) that increase in concentration accelerates the growth. After maximum growth the multiplication of bacteria decrease bringing down the transmittance to 46%, 44% and 41% at 144 hrs. (Tables, 3,4&5). In the curve it does not show toxic effect but definitely lead to toxicity when dosages are further increased and cessation of multiplication followed.

After 24 hrs in starch solution the organisms show rapid growth in early hours (Fig. 3) bringing down the transmittance to 25.6%, 24% and 22% in 0.1%, 0.2% and 0.3% of concentrations respectively. While this growth is decreased and percentage transmittance shoots upto 44%, 42% and 35% at 144 hrs, (Fig. 3 and Tables, 3,4&5).

Sucrose (Fig. 4) is also fermented easily and the maximum growth is noticed after 24 hrs with transmittance level to 34.6%, 25% and 24.6% in 0.1%, 0.2% and 0.3% of concentrations respectively. Further increase in dose from 0.1% to 0.3% does not have significant difference in the growth curves. The growth decreases to minimum after 96 hrs with 52%, 47.3% and 43% transmittance at 0.1%, 0.2% and 0.3% concentrations respectively (Tables, 3,4&5).

The growth of Klebsiella sp. in dextrose (Fig. 5) solution showed highest at 12 hrs with 36%, 28% and 27% transmittance in 0.1%, 0.2% and 0.3% in concentrations respectively. The growth decreased gradually throughout the experiment with slight fluctuations in growth curves at from 12 to 120 hrs. while in mannitol (Fig. 6) solution the Klebsiella sp. grows rather slowly and reaching highest growth (Fig. 6) after 36 hrs showing 35%, 32.6% and 28% transmittance in 0.1%, 0.2% and 0.3% concentrations respectively (Tables, 3,4&5). After that growth retards gradually throughout the experiment in all the concentrations.

EFFECTS OF VITAMINS :

All vitamins promote the growth of bacterium Klebsiella sp. upto some extent. Tremendous multiplication of bacterium (Klebsiella sp.) is noticed during 6 hrs to 12 hrs. After 24 hrs the growth decreases significantly in all the vitamins. It is interesting to note that in some cases (Vitamin B_{12}, E, K and C) the lag phase period of bacterium is enhanced.

The growth in vitamin B_{12} (Fig. 7) is very slow in early six hrs but after this the growth of organism starts rapidly reaching maximum after 24 hrs where the transmittance is 33%, 32% and 29% in 0.1%, 0.2% and 0.3% concentrations respectively. Thereafter the growth again decreases gradually upto 69%, 65% and 55% from transmittances after 144 hrs (Tables, 6,7&8).

The results obtained from vitamin B complex (Fig. 8) are the same as those with vitamin B_{12}. Highest growth is observed after 24 hrs with transmittances at 33%, 32% and 29% in 0.1%, 0.2% and 0.3% concentrations respectively. The growth is gradually decreased showing 69%, 65% and 55% transmittances at 144 hrs. Further increase in dose does not have any significant effect on the Klebsiella sp. (Tables, 6,7&8).

In vitamin K (Fig. 9) the highest growth is noticed after 12 hrs with 38.3%, 37% and 35% transmittance in 0.1%, 0.2% and 0.3% concentrations respectively. The growth again decreases throughout the experiment and minimum growth is recorded after 14 hrs showing 62.3%, 57.3% and 51.3% transmittances (Fig. 9). Like other vitamins, the vitamin C is also indispensable for the growth of Klebsiella sp. The highest growth with transmittance 45%, 32.6% and 42% is observed after 12 hrs. After 12 hrs the growth decreases gradually with few fluctuations in growth-curve. Further increase in concentrations has no significant effect (Fig. 10 and Tables, 6,7&8).

In vitamin A and vitamin E (Fig. 11 and Fig. 12) the growth is slightly affected. In both the cases the growth has been fast between 8 to 12 hrs of period after that the growth decreases. In vitamin A the maximum growth is noticed

after 24 hrs with 28%, 26.6% and 24.6% transmittances in 0.1%, 0.2% and 0.3% concentrations respectively. Similarly in vitamin E the highest growth is observed after 12 hrs with 35.6%, 31.6% and 28.3% transmittance (Tables, 6,7&8).

EFFECTS OF ANTIBIOTICS :

Althrocin, chloromycetin, terramycin, gentioyn, penicillin and streptomycin were used against the bacterium (Klebsiella sp.) and most of them were found growth inhibitor. Althrocin is found most effective followed by chloromycetin, terramycin and gentioyn, whereas penicillin and streptomycin are found least effective. During the study of effects of antibiotics marked variations in the growth curves have been observed and nearly in all the cases the multiplication period has been prolonged.

All the three concentrations of althrocin, 0.1%, 0.2% and 0.3% are equally effective. Growth of bacterium is gradually increased bringing the transmittance 50.6%, 50.6% and 52.3% in 0.1%, 0.2% and 0.3% concentrations respectively (Fig. 13). Later on, the growth is more or less constant or in other words the equilibrium is established. After 144 hrs the growth has been 51% transmittance in 0.1% conc. 51% in 0.2% and 57.3% in 0.3% thus highest being in 0.1% concentration and lowest in 0.3% concentration (Tables, 9,10&11).

In chloromycetin (Fig. 14) the growth increased upto 120 hrs showing 40.3%, 43.3% and 43.6% transmittances in 0.1%, 0.2% and 0.3% concentrations respectively. After 120 hr the growth is slightly decreased and finally at 144 hrs the transmittances are 41.6%, 44.0% and 46.6% in their respective concentrations (Tables, 9,10&11).

In terramycin, very slow and steady growth is observed (Fig. 15). The growth curves are very similar to those of chloromycetin. Highest growth is observed after 120 hrs with 22%,23.6% and 26.3% transmittances in 0.1%, 0.2% and 0.3% concentrations respectively. After 120 hrs. the growth tended to decrease bringing the transmittances to 21%, 26.6% and 29.6% at 144 hrs. (Tables, 9,10&11)

Similar results have been obtained with gentioyn (Fig. 16). The growth curve of gentioyn has been rather fluctuating. Growth has been slow reaching to 35.2%, 40.6% and 52.6% transmittances at 144 hrs.

In case of penicillin and streptomycin, the growth is rapid in both the antibiotics, thus both of them have been rather ineffective against the Klebsiella sp. (Fig 17&18) In penicillin maximum growth is observed after 144 hrs showing 19.3%, 21.3% and 22.3% transmittances (Fig. 17) in 0.1%, 0.2% and 0.3% concentrations respectively while

in streptomycin the growth reaches its peak after 36 hrs showing 23%, 32% and 40% transmittances at the above concentrations respectively. After 36 hrs the growth has been somewhat constant throughout the experiment (Tables, 9, 10&11).

EFFECTS OF INSECTICIDES :

DDT, BHC, sodium arsenate, zinc sulphate, calcium arsenate and Furadon, were tried against Klebsiella sp. Most effective insecticide has been DDT followed by BHC, sodium arsenate and zinc sulphate while calcium arsenate and Furadon are found to be least effective against Klebsiella sp.

All three concentrations of DDT, (0.1%, 0.2% and 0.3%) have bactericidal action. The growth has been rapid immediatel after inoculation upto 24 hrs showing 40%, 45.3% and 48% transmittance (Fig. 19). After 24 hrs the growth decreased, bringing the transmittance to 60.6%, 43.3% and 69.3% at 72 hrs in 0.1%, 0.2% and 0.3% of concentrations (Tables, 12,13 & 14)

In BHC (Fig. 20) the growth reaches its highest after 24 hrs with 42.3%, 45% and 58% transmittances. After 24 hrs the growth is nearly constant throughout the experiment. (Tables, 12, 13 & 14)

In sodium arsenate (Fig. 21) the growth has been slow, reaching maximum after 96 hrs with 30.6%, 39.3% and 30% transmittances in 0.1%, 0.2% and 0.3% concentrations respectively. A marked fluctuation has been noticed after 72 hrs especially in 0.3% concentration showing highest bactericidal action. After 96 hrs growth also tends to decrease. (Tables, 12, 13 & 14)

In zinc sulphate (Fig. 22) the growth is slow reaching maximum after 72 hrs bringing down the transmittances to 47.3%, 41% and 23%. The growth curves show increase in growth after 72 hrs and again a decline in last few hrs of growth (Tables, 12, 13 & 14).

Calcium arsenate and Euradon show the least bactericidal properties against the Klebsiella sp. In calcium arsenate the growth is slow (Fig. 23) but with varied fluctuations both inclination and declination in the growth curve (Tables, 12, 13 & 14) while in Euradon the maximum growth in recorded after 24 hrs showing 35.6%, 34.3% and 32.3% transmittances (Fig. 24). After 24 hrs the growth suddenly decreases upto 72 hrs bringing transmittances to 49.3%, 57% and 54% in 0.1%, 0.2% and 0.3% concentrations respectively (Tables, 12, 13 & 14).

D I S C U S S I O N

DISCUSSION

The rapid advancement in the microbial control of insects in various parts of the world has provided ample scope for utilisation of micro organisms both symbiotic and pathogenic for managing the pest population. To-day microbial control continued to depend on two methods of utilisation of microorganisms for suppression of the pest species. One method includes use of symbiotic micro-organisms by upsetting their balance existing in between the two (Brues & Dunn, 1945 and Khan, 1974,77,) while another involves the introduction of entomogenous bacteria which induce diseases in the hosts (Steinhaus, 1957a; Bucher, 1958, 1960 and Dutky, 1959). Both methods are equally effective, comparatively inexpensive and they have edge over chemical control in being rather specific and nontoxic to the mammals.

It is a well established fact that insects harbour microorganisms in some specialised tissues of the alimentary canal the mycetomes and a single cell is referred to mycetocytes. The microorganisms have been referred to as bacteroids or symbionts (Musgrave, 1964; Steinhaus, 1967 and Khan, 1973). However, to-day these microorganisms have been cultured on various artificial media *in vitro*.

These bacteria are identified on the basis of morphological and biochemical tests.

In the present investigations attempts have been made to find out the presence of mycetome and mycetomal microorganisms in C. punctata Walk. an agriculturally important pest. Extensive study has been made on its morphology and anatomy.

Microorganisms have been isolated and identified as accurately as possible. In these observations Klebsiella sp. is isolated and identified from the mycetomes of C. punctata.

The organism is capable of growing on different artificial media, i.e., nutrient agar gelatin stab and nutrient broth. It is rod shaped, non motile, occurring singly or in pairs encapsulated and gram negative. These findings are, therefore, in accordance with those of Khan's (1976) where he also found association of a gram negative bacterium with the mycetome of Idaeocerus clypealis Leth.

The disc diffusion test for sensitivity showed that the growth of the bacterium is highly inhibited by chloromycetin and althrocin followed by penicillin, gentiocyn and terramycin. It is interesting to note that this bacterium shows least sensitivity to streptomycin. Results show the similarity with those obtained by Khan (1974 & 1976) on

bacterial symbionts from Dysdercus cingulatus and Pyrilla perpusilla Walk. and Brues & Dunn (1945) on intracellular bacteriods of cockroaches.

The studies on different concentrations (0.1%, 0.2% and 0.3%) of sugar, vitamins, antibiotics and insecticides on the growth of Klebsiella sp. in vitro show that nearly all sugars (sucrose, lactose, maltose, dextrose, mannitol and starch) and vitamins (vitamn A, vitamin B_{12}, vitamin C, vitamin B complex, vitamin K and vitamin E) have been found promoting and accelerating the growth of the bacterium. In other words, it can be safely concluded that sugars and vitamins both work as growth factors. Similar conclusions were obtained by Corner & Hansen (1967) with amino acids, B-vitamins, purines, pyrimidines nucleotides nucelosides and coenzymes.

All the concentrations of sugars (0.1%, 0.2% and 0.3%), tried against Klebsiella sp. show the increased growth in high concentration of sugars, i.e., 0.3%, while average growth is noticed in 0.2% and least growth in 0.1% concentration. It is interesting to note that when the concentrations increased growth retards. Thick growth is observed in maltose, lactose, starch and sucrose bringing down the transmittance to 24%, 24% and 21%, (24%, 27% and 25%); (25.6%, 24%, and 22%) and (34.6%, 25% and 24.6%) in 0.1%, 0.2% and 0.3% of concentrations of sugars respectively while other sugars result in poor growth of Klebsiella sp.

The multiplication of Klebsiella sp. is noticed during 8-12 hrs in all the vitamins except vitamin A and vitamin E, where growth is not so rapidly promoted. In vitamin B_{12} and vitamin B complex the highest growth is observed after 24 hrs (33%, 32%, 29%) (33%, 32%, 29%) and 33%, 32% and 29% while in vitamin K and vitamin C the highest growth is observed after 12 hrs with transmittances 38.3%, 37%, 35% and (45%, 32%, 42%). In case of vitamin A and vitamin E the growth is not so rapid. Dias & Bhat (1964) found similar results showing necessity of fatty acids and vitamin K for Rumen bacteria. Atkinson & Robinson (1935) found that vitamins and amino acids act as growth factors for certain bacteria and other microbiota. It is noticed during the growth of organism that it is able to grow upto some period and later the growth declines and after some time it again starts to grow more. It may be concluded that bacterium has produced some toxic substances during metabolism which cause inhibition of growth. After some time toxic substances are neutralised and the bacteria again start growing (Steinhaus & Birkeland 1939).

All the antibiotics in different concentrations (0.1%, 0.2% and 0.3%) inhibited the growth. Highly satisfactory results have been obtained by chloromycetin,

althrocin, penicillin, gentioyn and terramycin while streptomycin is least effective. These results confirmed the views of Brooks & Richards (1955) and Bruss & Dunn (1945), where they were successful in eliminating the mycetomal symbionts of Blatta germanica through antibiotics and sulpha drugs.

Althrocin shows the highest growth with 50.6%, 50.6% and 52.3% transmittances at 36 hrs, and chloromycetin 40.3%, 43.3% and 43.6% transmittances at 120 hrs. Highest growth in terramycin and gentioyn ranges from 26-33.3% and 35.2-52.6% transmittances respectively, while in case of penicillin and streptomycin highest growth ranges from 19-22.3% and 23-40% transmittances respectively. These results further strengthen the finding of Koch (1936a and 1956) he was successful in inhibiting the bacterium in the fat bodies of Periplaneta americana.

DDT, BHC and sodium arsenate show highly bactericidal action against the Klebsiella sp. while others are less effective against this bacterium. These insecticides probably inhibit the growth by killing the enzyme system and damaging the cell organelles which results into death of the bacterium. All the concentrations of insecticides are effective. Highest growth is noticed in DDT and BHC after 24 hrs with 40%, 45.3%, 48.3% and 42.3%, 45%, 50% transmittances

respectively while in sodium arsenate and zinc sulphate highest growth with 30%, 37%, 30% and 45%, 40.6%, 25% respectively after 96 hrs while calcium arsenate and Furadon are least effective. Similar results were obtained by Hershberger & Forgash (1964a & 1964b) on Periplaneta americana by using lindane solution through feed. He found lindane was highly effective against intestinal and intracellular organisms.

The control of insects faces on the one hand great concern about environmental pollution by poisons and on the other hand the resistance of pests, developed against the insecticides. Consequently alternatives to chemical control are being urgently needed. Among most interesting is utilisation of insect microorganisms entomogenous or symbiotic or both. The present studies give ample proof that these microorganisms are potential control measures of the future.

TABLE- 1

DETAILS OF BIOCHEMICAL REACTIONS OF THE MICRO-ORGANISM ISOLATED FROM THE INTESTINES OF *Clovia puncta* Walker

Sl. No.	Name of organism	Substrate	Reactions
1.		Indol	(-)
2.		Hydrogen sulphide	(-)
3.		Litmus milk	(+)
4.		Methyl red	(+)
5.		Acetyle methyle carvinol	(+)
6.		Blood Agar	(-)
7.		Nitrate	(+)
8.	*Klebsiella* sp.	Fructose	(+)A
9.		Gluose	(+)AG
10.		Sucrose	(+)AG
11.		Lactose	(+)
12.		Dextrose	(+)A
13.		Mannitol	(+)
14.		Galactose	(+)

(-) = Negative
(+) = Positive
(A) = Acid
(AG) = Acid & Gas.

TABLE - 2

SENSIVITY TEST OF DIFFERENT ANTIBIOTICS AGAINST *Klebsiella* sp.

Sl. No.		Zone of inhibition (mm)
1.	Chloromycetin	32
2.	Althrocin	30
3.	Penicillin	30
4.	Gentioyn	25
5.	Terramycin	20
6.	Streptomycin	12

Each antibiotics was used in 0.1% concentration.

TABLE - 3

EFFECT OF 0.1% CONCENTRATION OF DIFFERENT SUGARS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Sugar	Total Growth % Transmittance	Mean	S. Deviation	S. Error
1.	Maltose	545.9	30.99	17.99	± 4.5
2.	Lactose	600.5	43.46	17.16	± 4.58
3.	Starch	601.4	42.95	16.72	± 4.47
4.	Sucrose	602.8	47.34	15.10	± 4.03
5.	Dextrose	679.2	48.51	14.79	± 3.95
6.	Mannitol	722.6	15.6	14.03	± 3.75

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittence.

TABLE - 4

EFFECT OF 0.2% CONCENTRATION OF DIFFERENT SUGARS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Sugar	Total growth % Transmittence	Mean	S. Deviation	S. Error
1.	Maltose	522.1	37.29	17.35	± 4.63
2.	Lactose	575.6	41.11	16.99	± 4.54
3.	Starch	557.7	39.83	10.94	± 2.92
4.	Sucrose	618.1	44.15	16.10	± 4.30
5.	Dextrose	628.8	44.91	14.67	± 3.92
6.	Mannitol	661.0	47.21	14.29	± 3.82

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittence.

TABLE - 5

EFFECT OF 0.3% CONCENTRATION OF DIFFERENT SUGARS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Sugar	Total growth % Transmittance	Mean	S. Deviation	S. Error
1.	Maltose	469.5	33.57	16.27	± 4.35
2.	Lactose	555.7	39.69	16.9	± 4.54
3.	Starch	499.5	35.67	15.8	± 4.22
4.	Sucrose	566.4	40.45	14.95	± 3.9
5.	Dextrose	581.3	41.52	13.74	± 3.67
6.	Mannitol	616.0	44.0	15.04	± 4.02

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 6

EFFECT OF 0.1% OF DIFFERENT CONCENTRATION OF VITAMINS ON THE GROWTH OF **Klebsiella** sp. **in vitro**

Sl. No.	Name of Vitamin	Total growth in % transmittance	Mean	S. Deviation	S. Erro
1.	Vitamin B12	840.0	60.04	18.37	± 4.91
2.	Vitamin B Complex	800.0	57.14	13.12	± 3.5
3.	Vitamin K	805.4	57.52	18.09	± 4.83
4.	Vitamin C	812.2	58.01	17.28	± 4.62
5.	Vitamin A	719.6	51.4	18.81	± 5.02
6.	Vitamin E	833.9	59.56	17.64	± 4.71

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittence.

TABLE - 7

EFFECT OF 0.2% OF VITAMINS ON THE GROWTH OF **Klebsiella** sp. **in vitro**

Sl. No.	Name of Vitamin	Total growth in % transmittance	Mean	S. Deviation	S.Error
1.	Vitamin B12	797.13	56.95	18.36	± 4.9
2.	Vitamin B complex	731.7	52.26	18.30	± 4.8
3.	Vitamin K	691.9	49.42	22.60	± 6.04
4.	Vitamin C	744.6	53.18	19.17	± 5.12
5.	Vitamin A	616.4	44.02	20.90	± 5.59
6.	Vitamin E	767.1	56.22	18.12	± 4.84

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE- 8

EFFECT OF 0.3% OF DIFFERENT CONCENTRATION OF VITAMINS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Vitamin	Total growth in % Transmittance	Mean	S. Deviation	S. Error
1.	Vitamin B12	758.0	54.14	18.85	± 5.04
2.	Vitamin B complex	669.2	47.8	19.18	± 5.12
3.	Vitamin K	613.0	43.80	26.24	± 6.99
4.	Vitamin C	648.3	46.30	20.10	± 5.37
5.	Vitamin A	520.70	37.19	21.09	± 5.63
6.	Vitamin E	765.7	54.69	18.53	± 4.95

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 9

EFFECT OF 0.1 % CONCENTRATION OF DIFFERENT ANTIBIOTICS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Antibiotics	Total Growth % Transmittance	Mean	S. Deviation	S. Error
1.	Althrocin	821.7	58.69	18.66	± 4.98
2.	Chloromycetin	643.8	60.27	18.28	± 4.88
3.	Terramycin	809.8	57.84	25.40	± 6.79
4.	Gentiayn	722.2	51.58	21.46	± 5.73
5.	Penicillin	564.2	40.3	13.03	± 3.48
6.	Streptomycin	522	37.28	23.6	± 6.31

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE- 10

EFFECT OF 0.2% CONCENTRATION OF DIFFERENT ANTIBIOTICS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Antibiotics	Total growth in % transmittance	Mean	S. Deviation	S.Error
1.	Althrocin	847.6	60.54	15.16	± 4.05
2.	Chloromycetin	799.90	57.13	23.04	± 6.16
3.	Terramycin	851.90	60.85	25.63	± 6.80
4.	Gentiayn	808.70	57.76	15.93	± 4.25
5.	Penicillin	601.90	42.90	27.46	± 7.30
6.	Streptomycin	600.20	42.90	21.50	± 5.74

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 11

EFFECT OF 0.3% CONCENTRATION OF DIFFERENT ANTIBIOTICS ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl No.	Name of Antibiotics	Total Growth % Transmittance	Mean	S. Deviation.	S. Error
1.	Althrocin	861.19	61.56	15.42	± 4.12
2.	Chloromycetin	895.20	63.94	18.01	± 4.81
3.	Terramycin	939.20	67.08	24.40	± 6.50
4.	Gentisyn	903.0	64.5	16.48	± 4.40
5.	Penicillin	633.3	45.23	26.50	± 7.0
6.	Streptomycin	659.6	47.11	24.46	± 6.54

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 12

EFFECT OF 0.1 % CONCENTRATION OF DIFFERENT INSECTICIDES ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Insecticide	Total growth in % transmittance	Mean	S.Deviation	S. Error
1.	DDT	732.8	52.34	16.90	± 4.51
2.	BHC	700.80	50.05	17.60	± 4.70
3.	Sod. Arsenate	712.70	50.07	21.73	± 5.81
4.	Zinc Sulphate	841.70	60.12	20.52	± 5.48
5.	Calcium Arsenate	707.7	50.55	22.76	± 6.08
6.	Furadon	760.0	54.28	16.47	± 4.4

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 13

EFFECT OF 0.2% CONCENTRATION OF DIFFERENT INSECTICIDES ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Insecticide	Total growth % Transmittance	Mean	S. Deviation	S. Error
1.	DDT	800.2	57.15	15.38	± 4.11
2.	BHC	775.0	53.35	16.57	± 4.43
3.	Sod. Arsenate	788.3	56.30	20.72	± 3.74
4.	Zinc sulphate	812.3	58.02	23.92	± 6.39
5.	Calcium arsenate	727.3	51.95	23.89	± 6.38
6.	Furadon	752.3	53.73	6.50	± 1.73

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

TABLE - 14

EFFECT OF 0.3% CONCENTRATION OF DIFFERENT INSECTICIDES ON THE GROWTH OF *Klebsiella* sp. *in vitro*

Sl. No.	Name of Insecticide	Total growth in % transmittance	Mean	S. Deviation	S. Error
1.	DDT	897.7	64.12	16.77	± 4.4
2.	BHC	832.30	59.45	15.52	± 4.14
3.	Sod. Arsenate	843.0	60.21	20.00	± 5.35
4.	Zinc Sulphate	428.0	44.85	28.95	± 7.7
5.	Calcium Arsenate	750.8	53.62	24.22	± 6.47
6.	Furadon	846.0	46.14	14.06	± 3.75

a - Each reading is a mean of three replicates.

b - Observation period 0-144 hrs.

c - Interval from 0 hrs to every 12 hrs.

d - Growth was expressed in terms of % transmittance.

1-6. Effect of various concentrations of sugars on the growth of Klebsiella sp. (in terms of transmittance percentage) From 0.0 hr to 144 hrs.

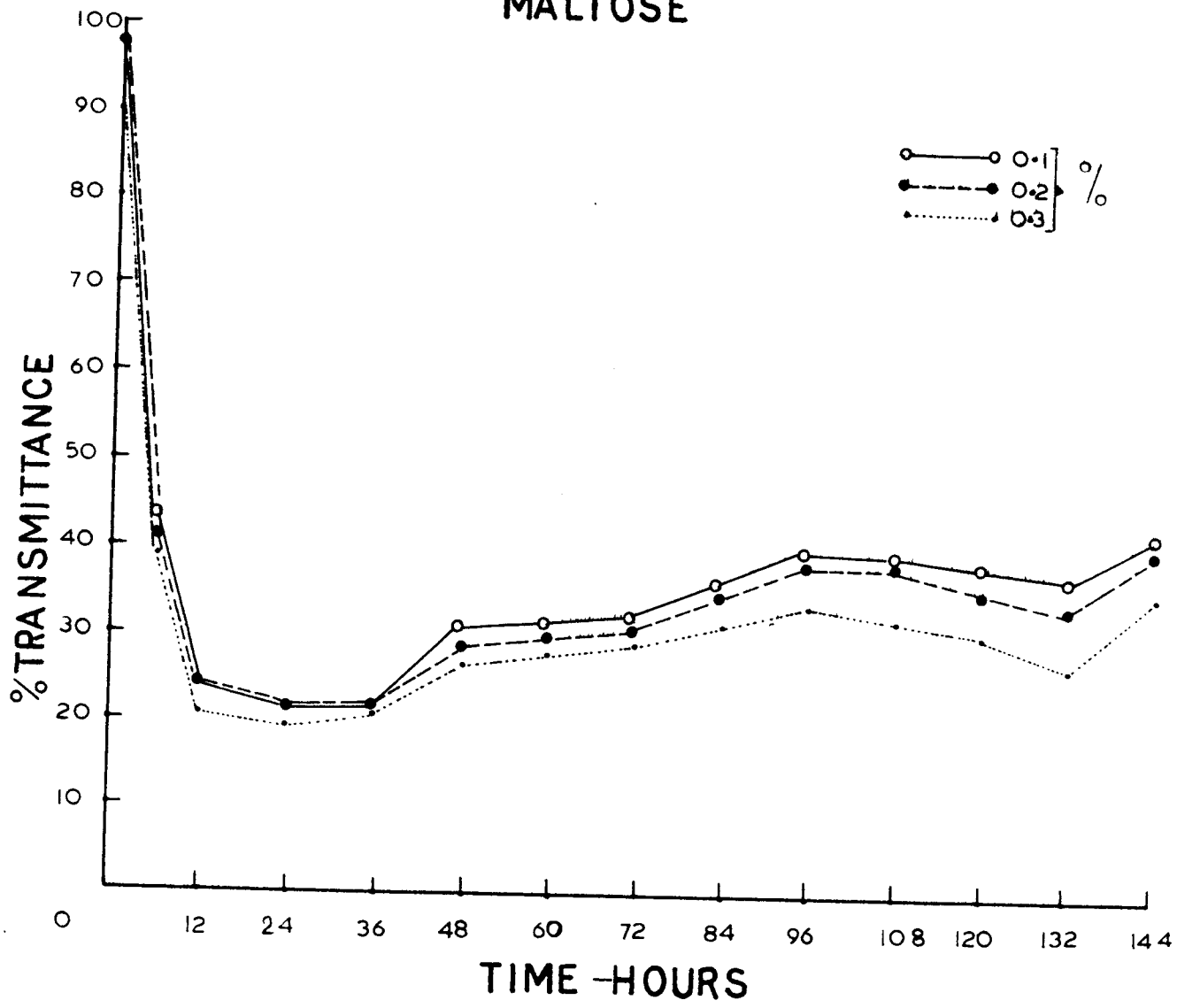

MALTOSE
100
90
80
70
60
50
40
30
20
10
0
% TRANSMITTANCE
0·1
0·2
0·3
%
12
24
36
48
60
72
84
96
108
120
132
144
TIME -HOURS

FIG. 1

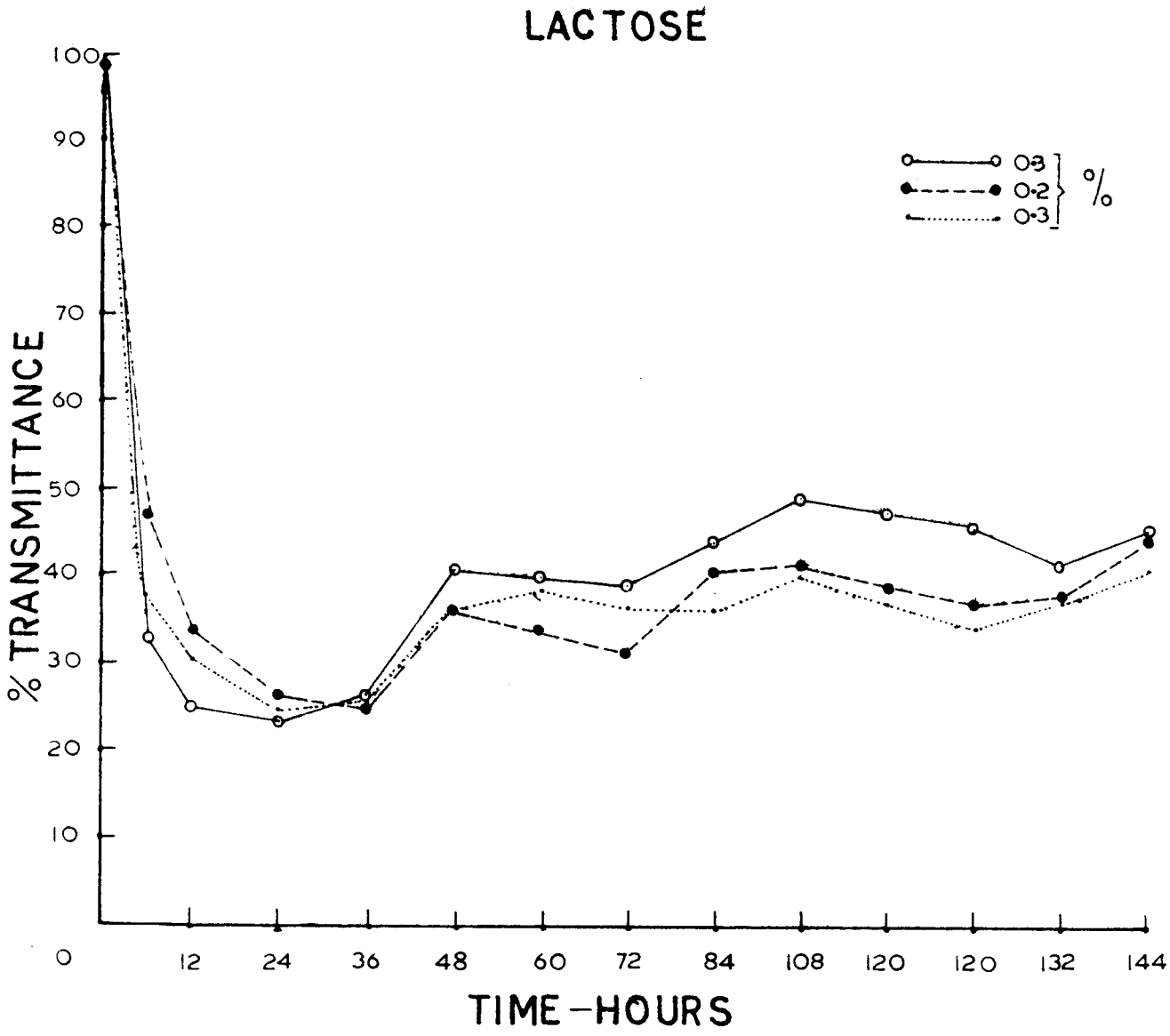

LACTOSE
100
90
80
70
60
50
40
30
20
10
0
% TRANSMITTANCE
0·1
0·2
0·3
%
12
24
36
48
60
72
84
108
120
120
132
144
TIME – HOURS

FIG 2

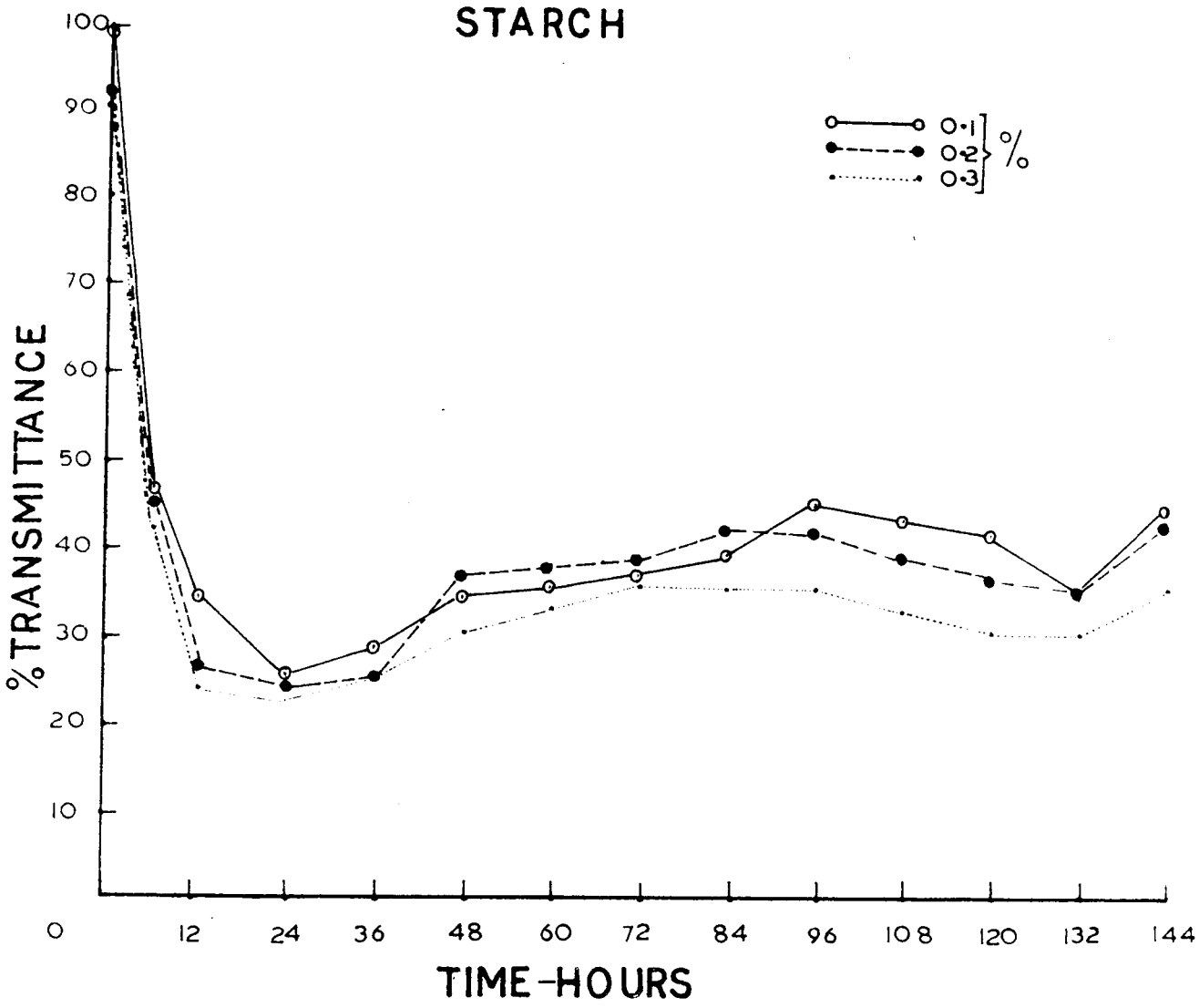
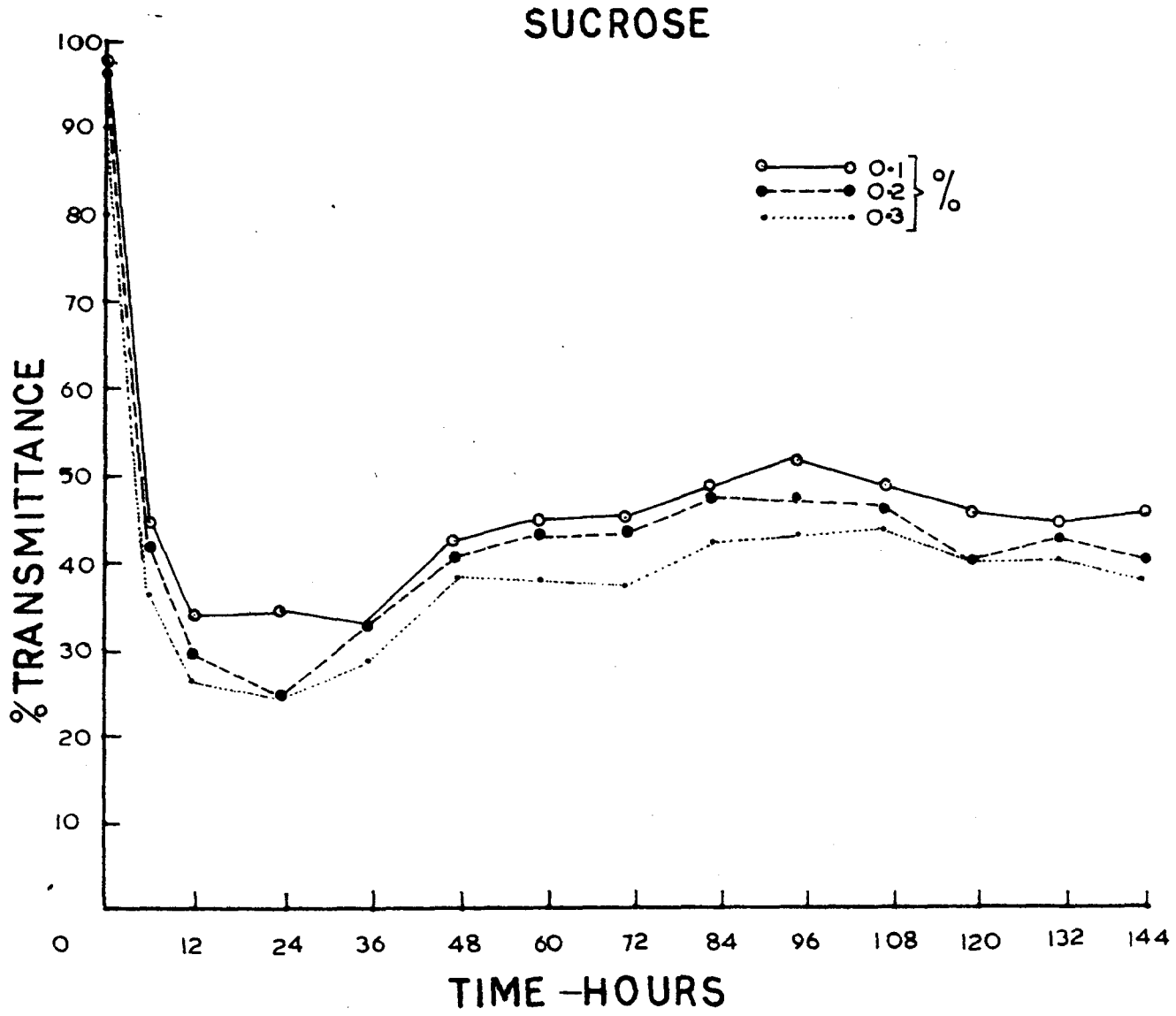
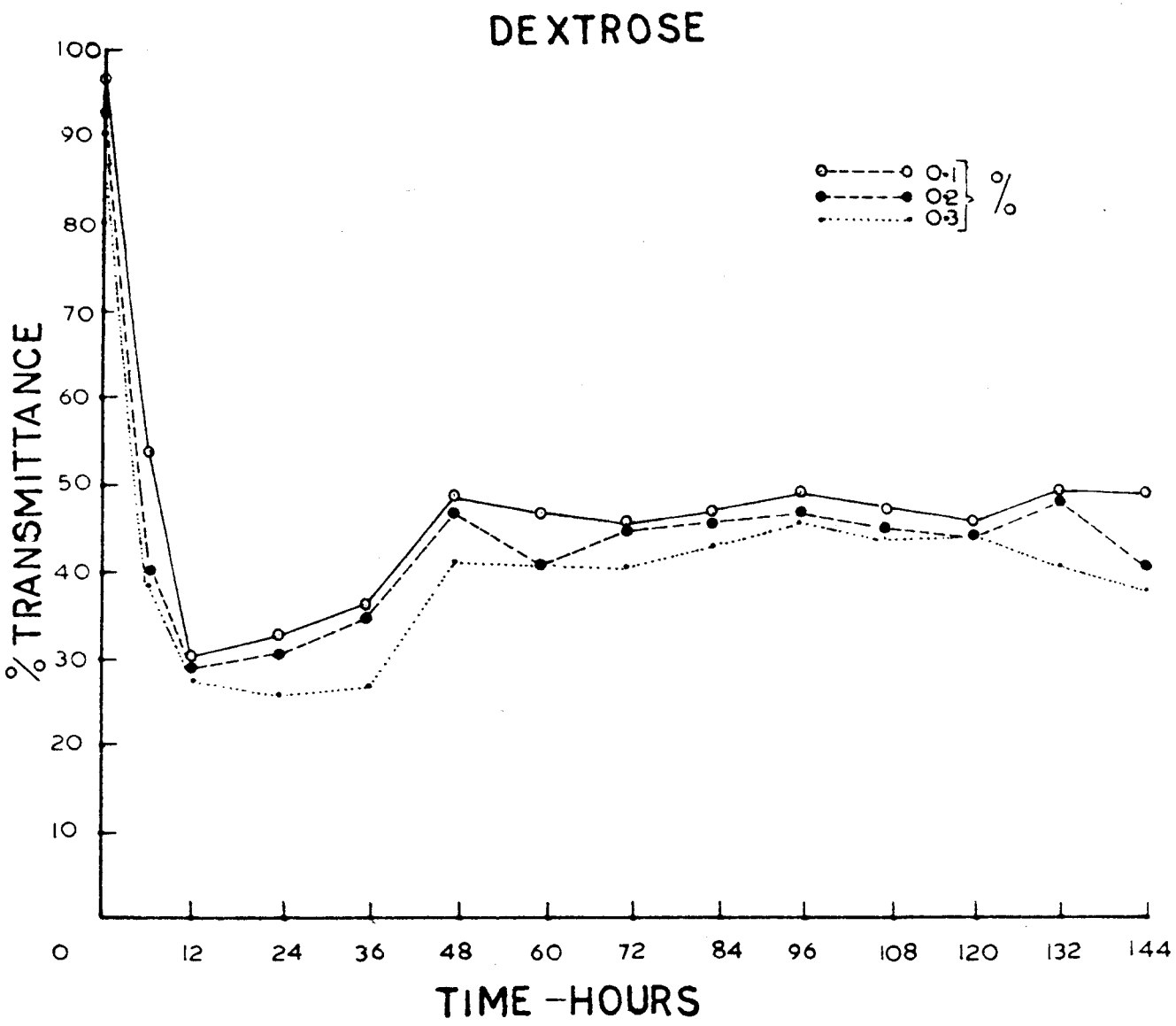

STARCH
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME-HOURS

FIG. 3


SUCROSE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
% TRANSMITTANCE
TIME –HOURS
0·1
0·2
0·3
%

FIG·4


DEXTROSE
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME - HOURS
0·1
0·2
0·3
%

FIG·5

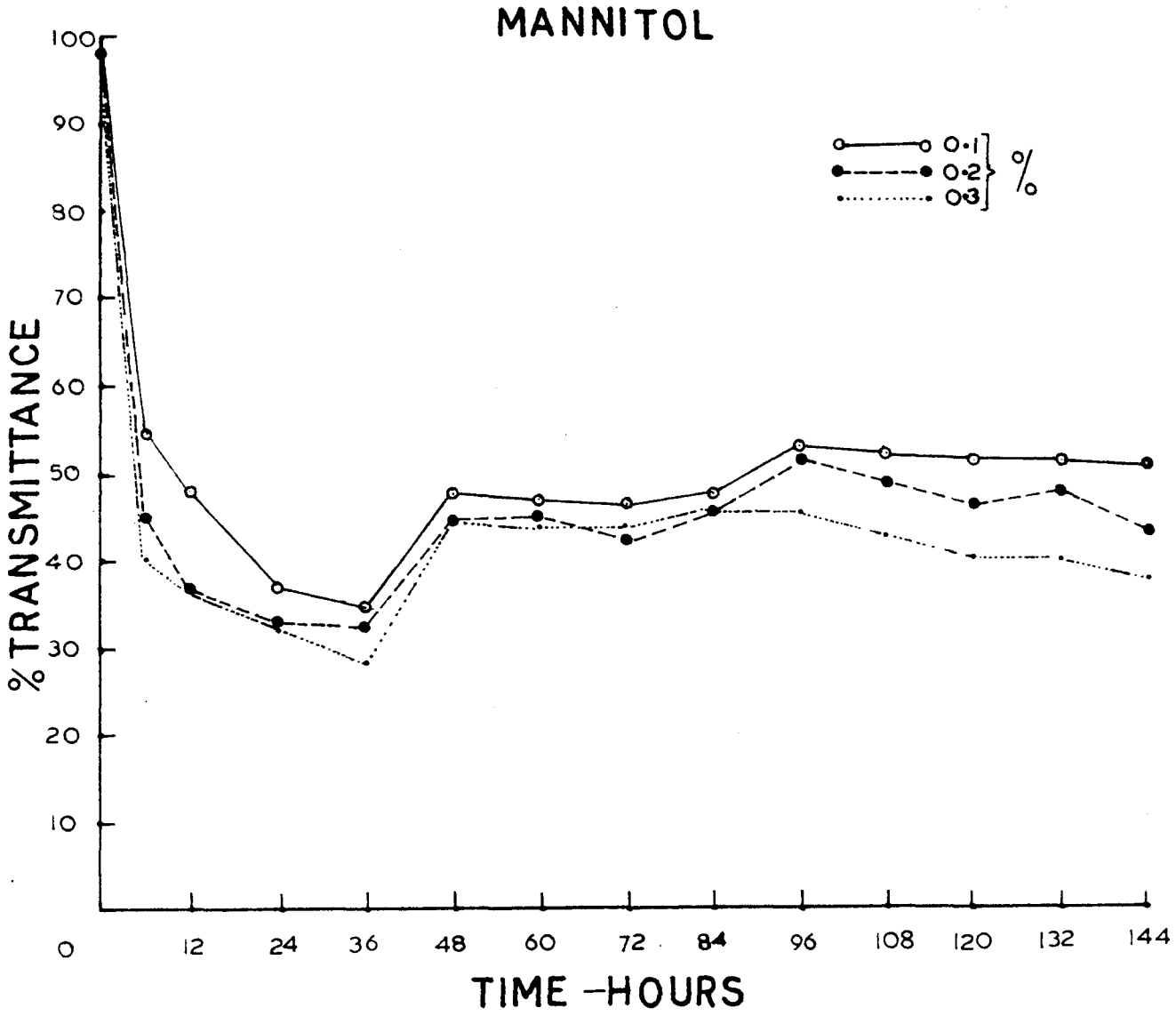

MANNITOL
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME -HOURS

FIG. 6

Figure 7 - 12. Effect of various concentrations of vitamins on the growth of Klebsiella sp. (in terms of transmittance percentage) From 0.0 hr. to 144 hrs.

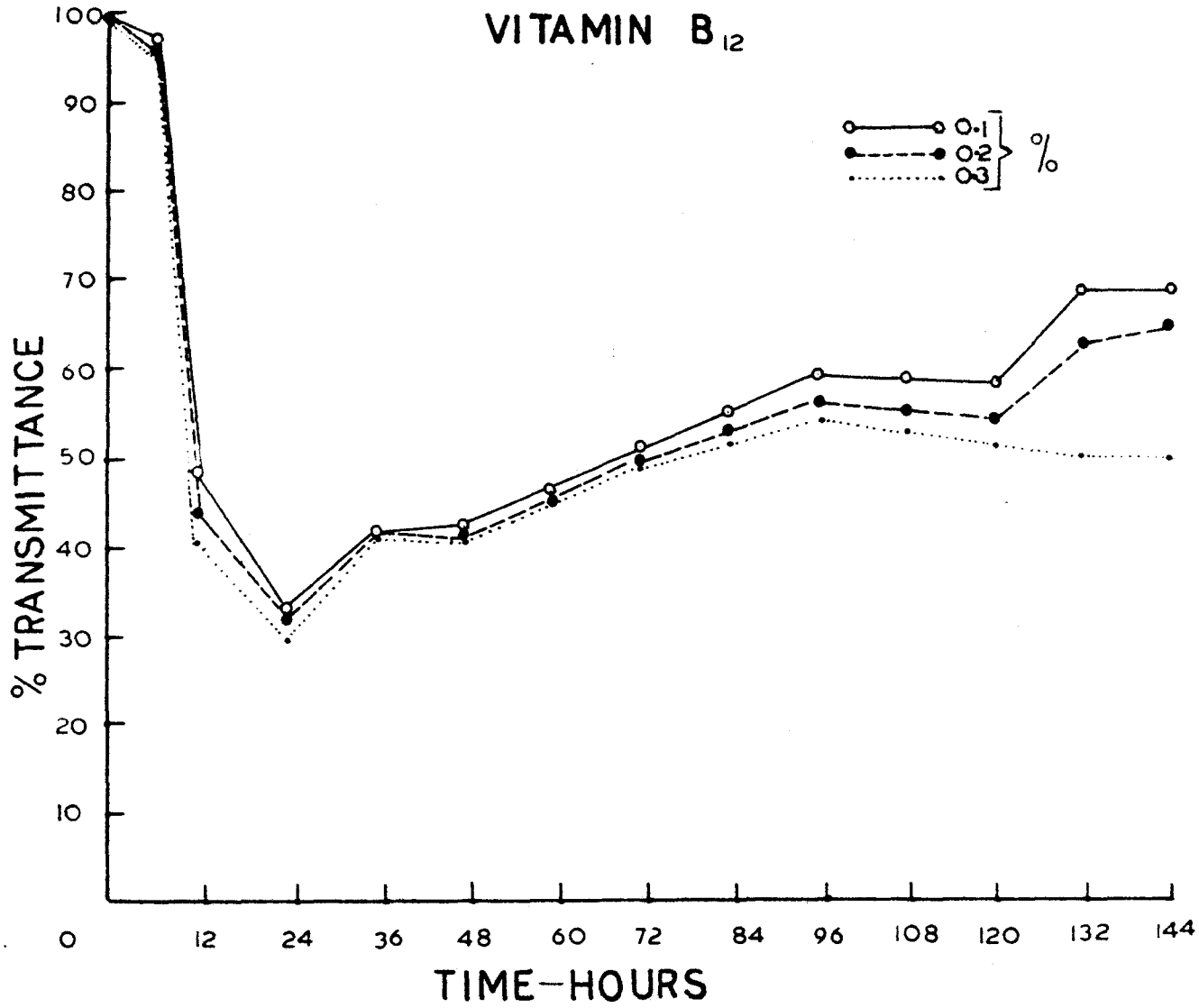
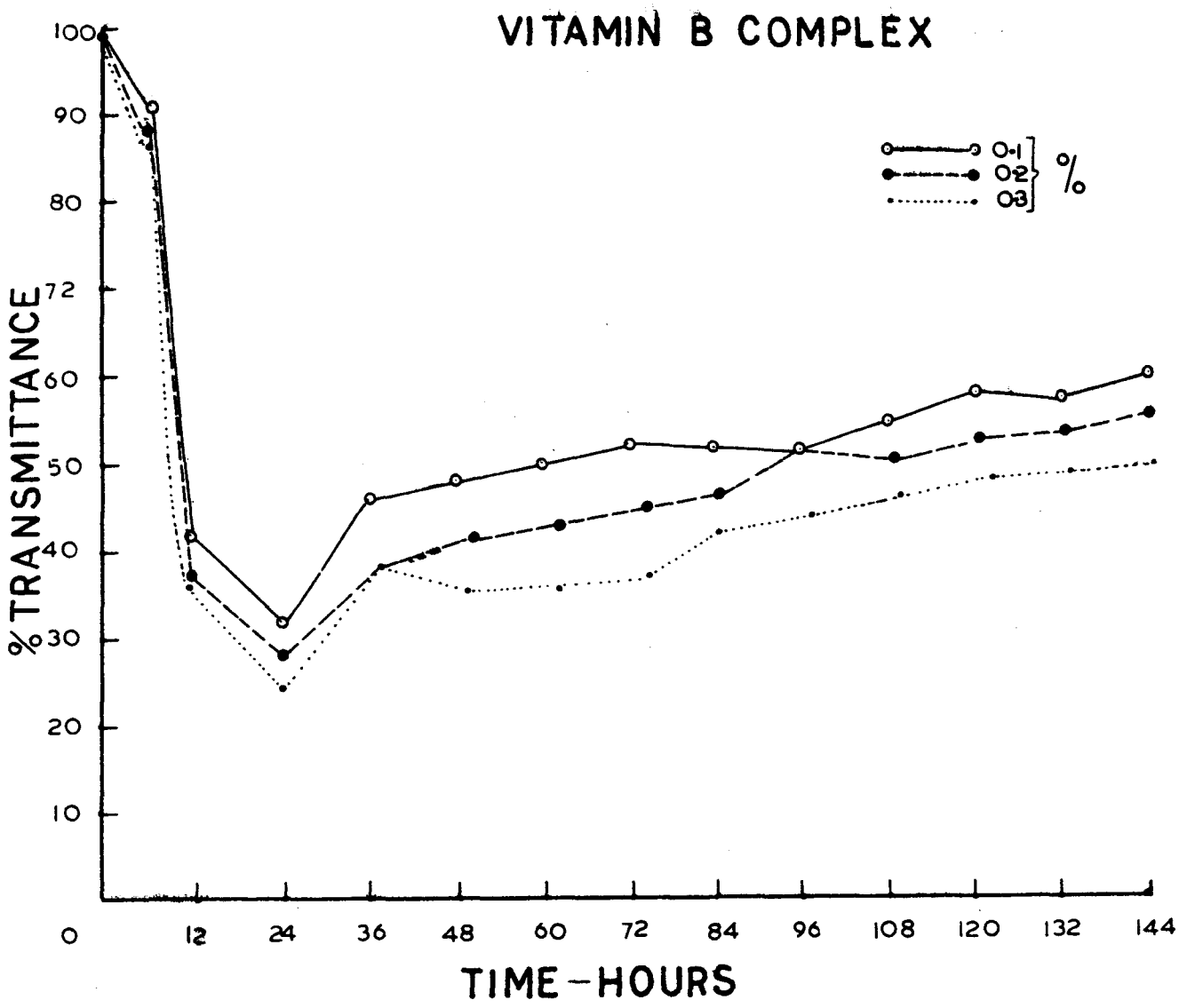
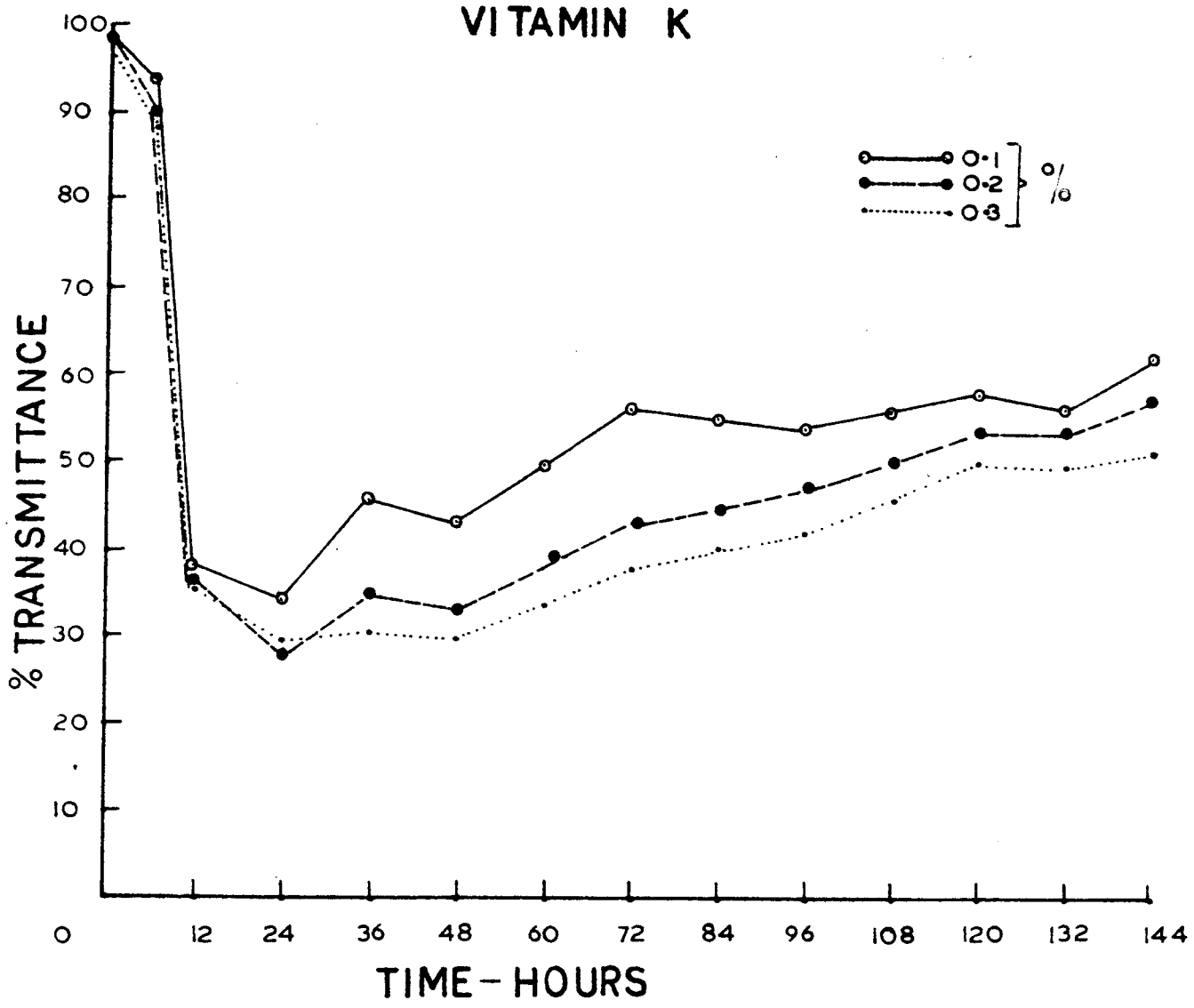

VITAMIN B12
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME–HOURS

FIG. 7


VITAMIN B COMPLEX
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
72
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME – HOURS

FIG·8


VITAMIN K
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME - HOURS
0·1
0·2
0·3
%

FIG·9

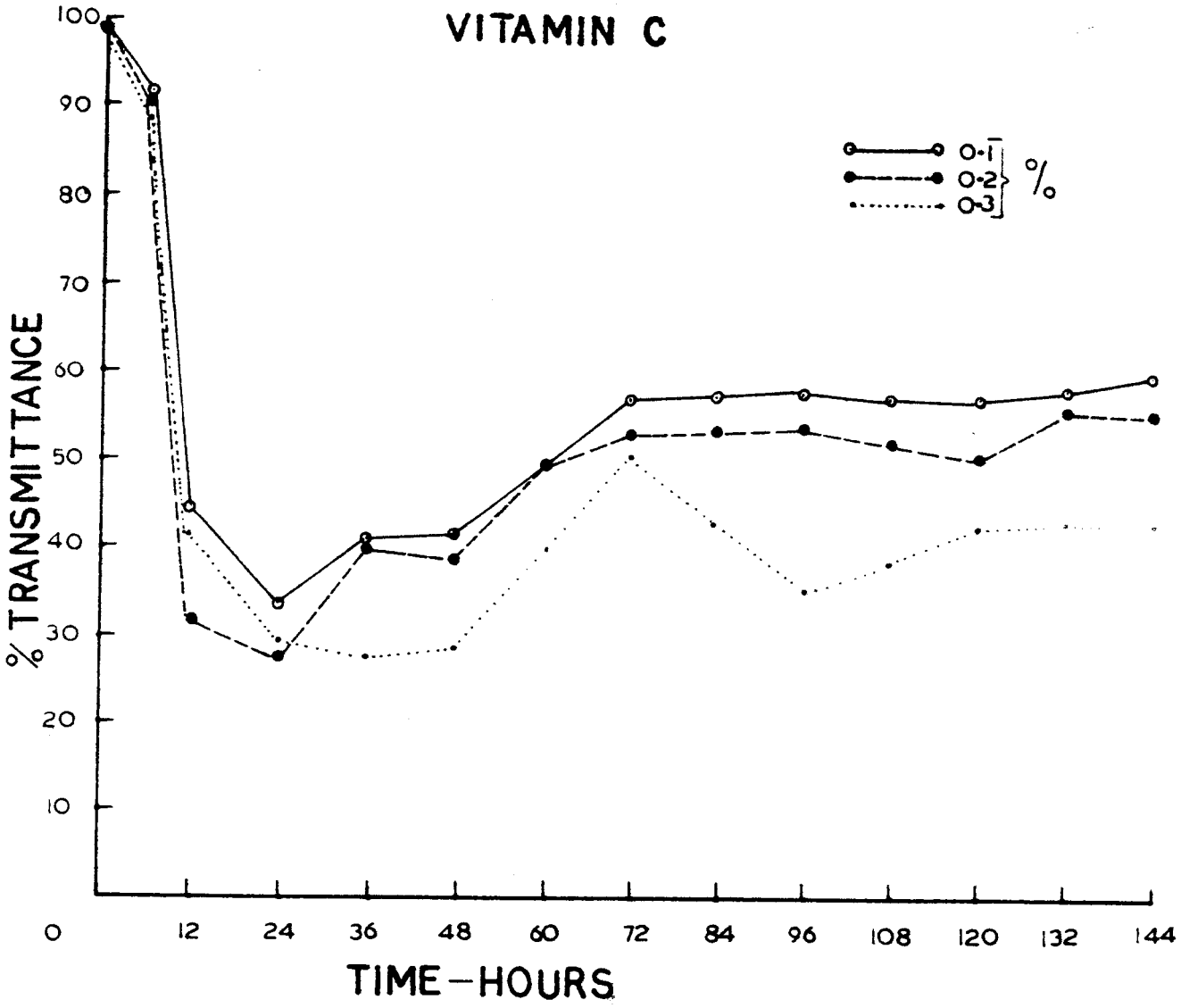

VITAMIN C
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME—HOURS

FIG. 10

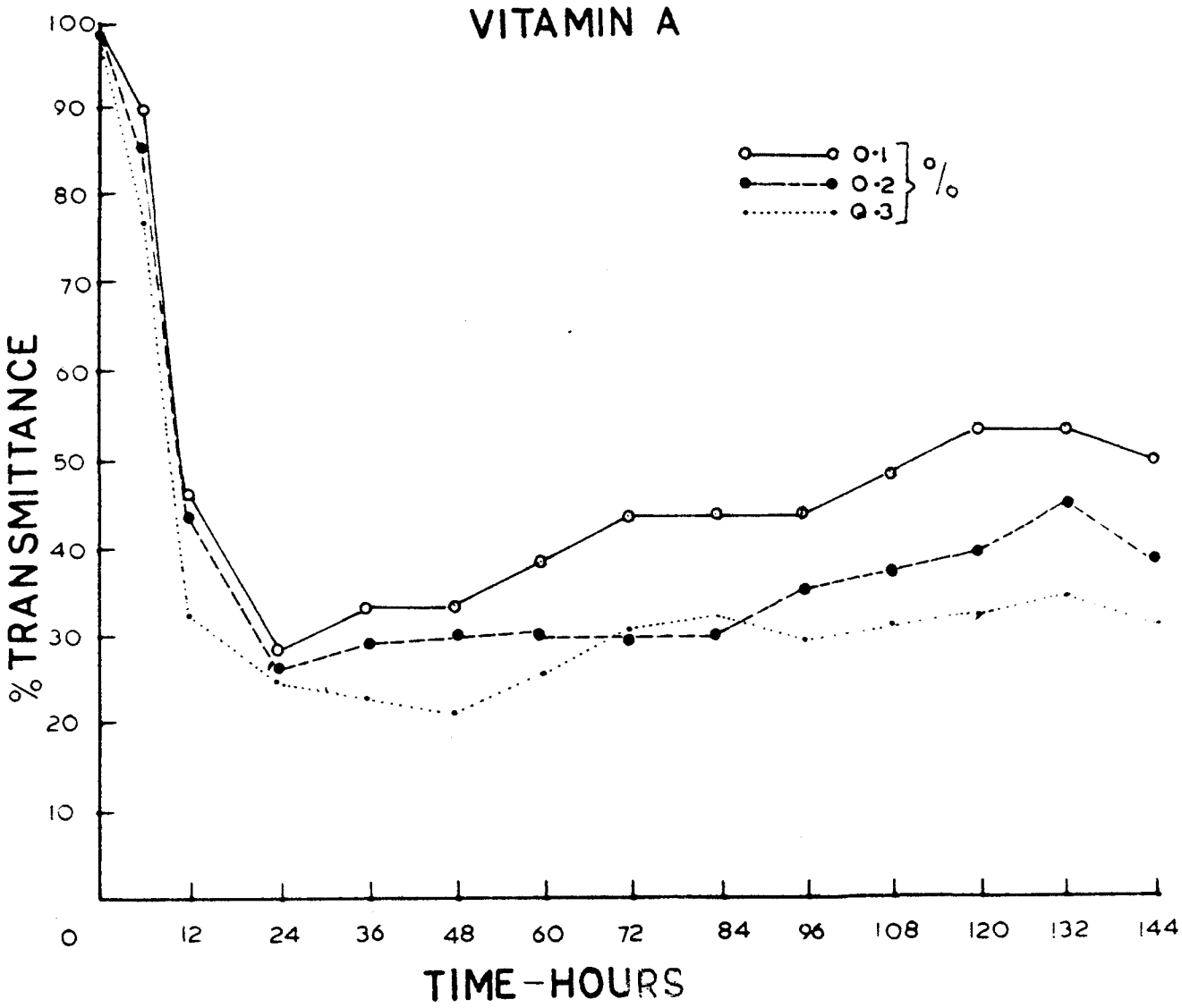

VITAMIN A
0·1
0·2
0·3
%
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME – HOURS

FIG. 11

Figure 13-18. Effect of various concentrations of antibiotics on the growth of *Klebsiella* sp. (in terms of transmittance percentage) From 0.0 hr to 144 hrs.

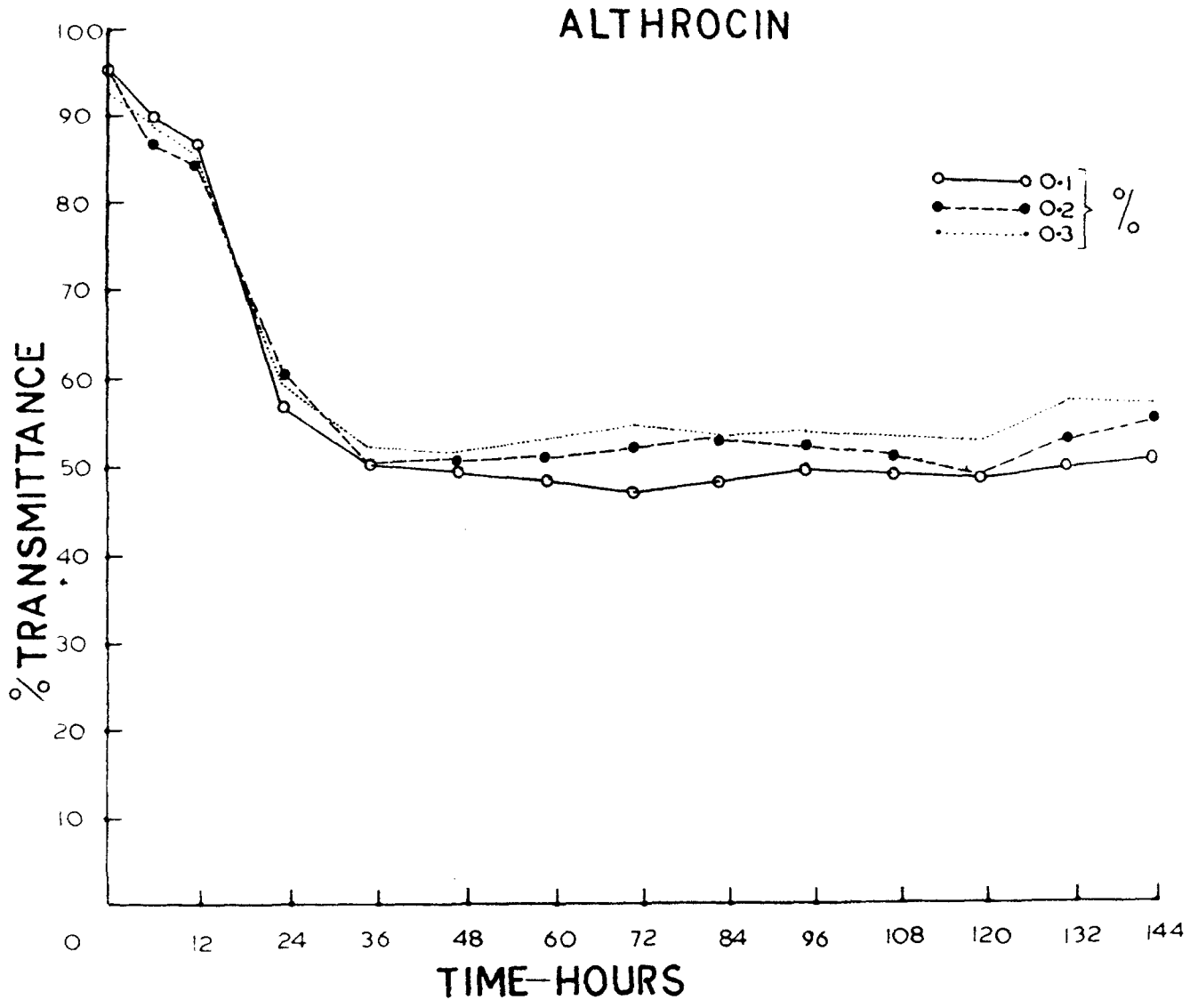

ALTHROCIN
100
90
80
70
60
50
40
30
20
10
0
% TRANSMITTANCE
0·1
0·2
0·3
%
12
24
36
48
60
72
84
96
108
120
132
144
TIME—HOURS

FIG. 13

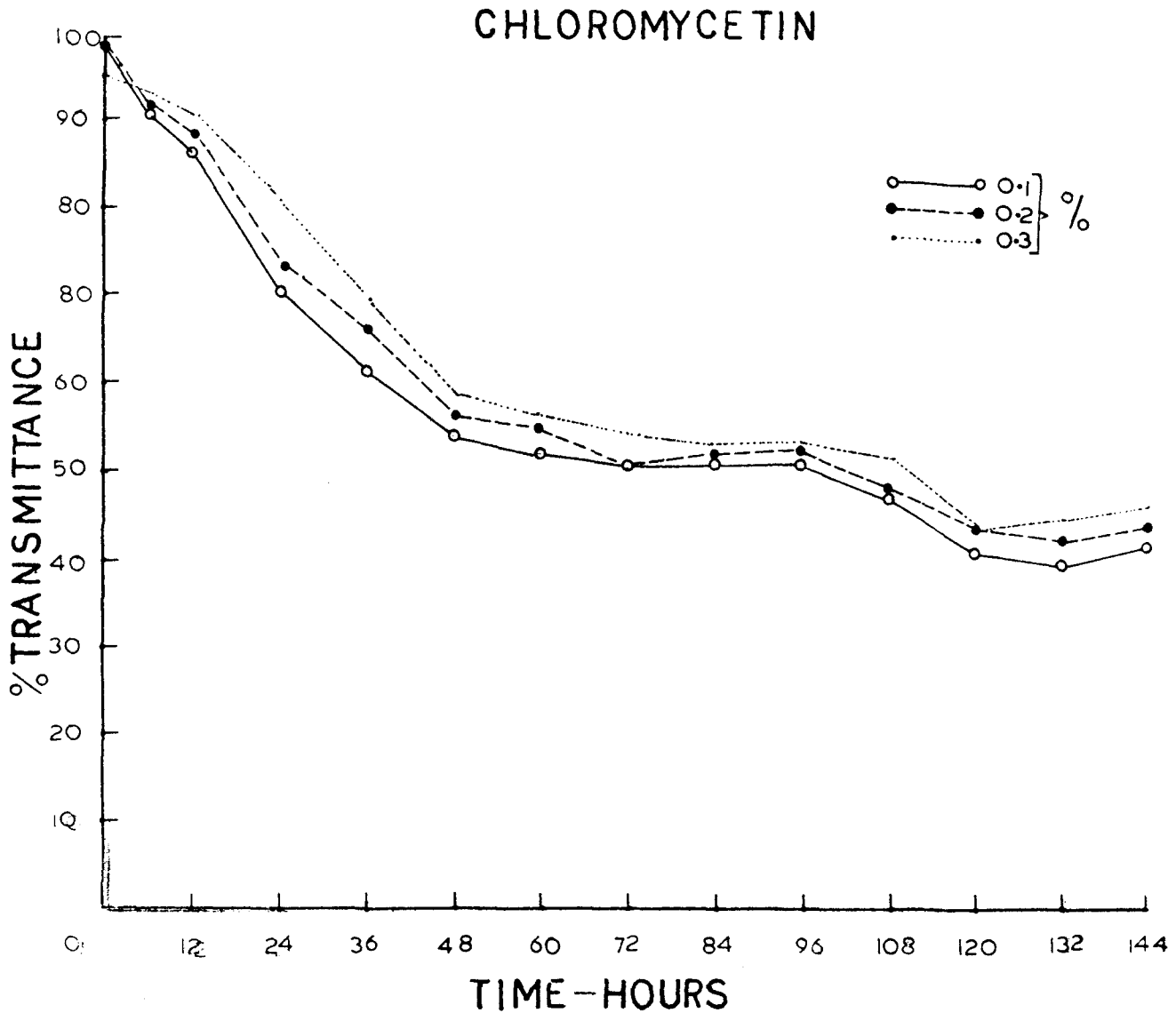

CHLOROMYCETIN
100
90
80
80
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
% TRANSMITTANCE
TIME – HOURS
0·1
0·2
0·3
%

FIG. 14

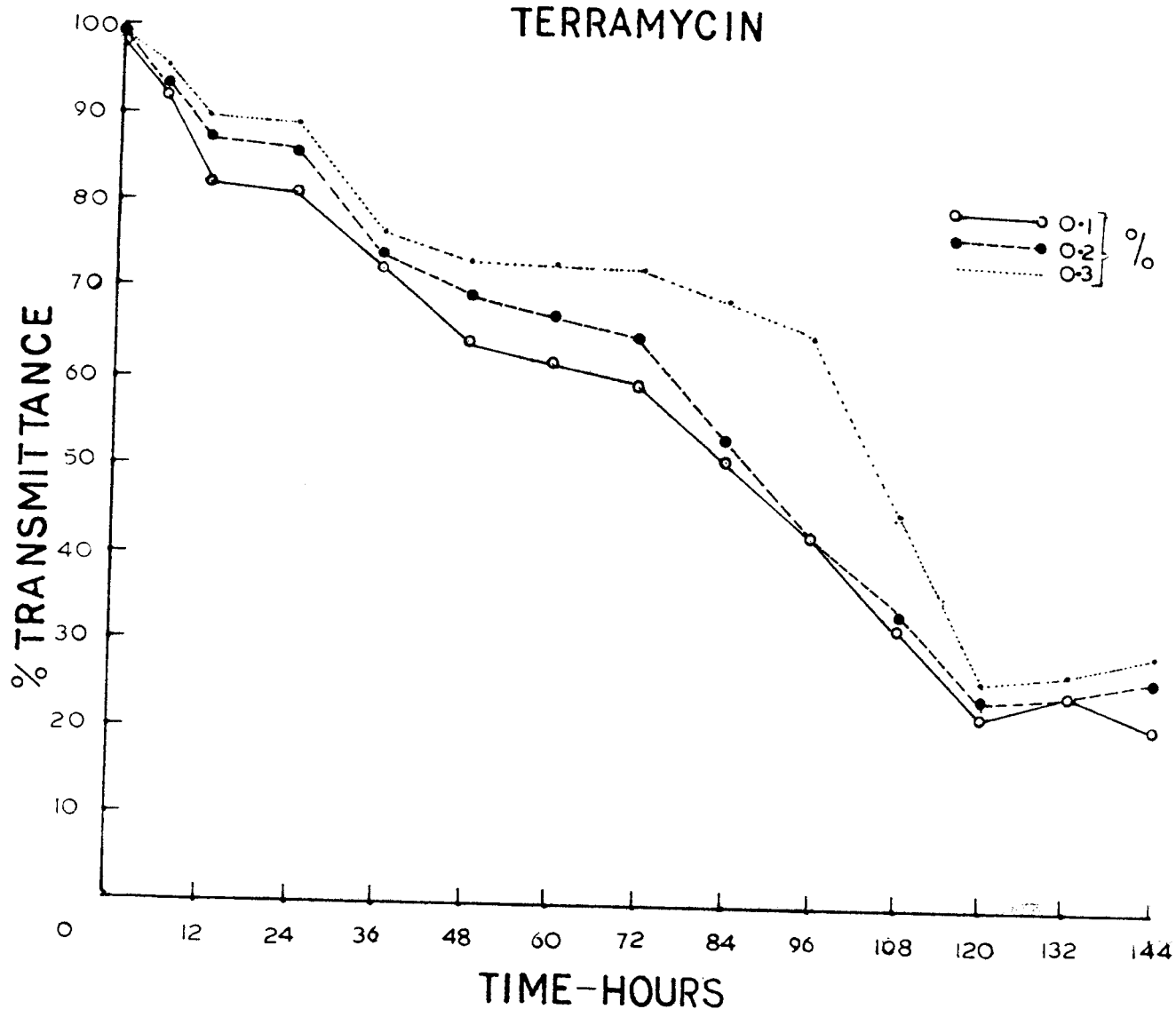

TERRAMYCIN
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME-HOURS
0·1
0·2
0·3
%

FIG. 15

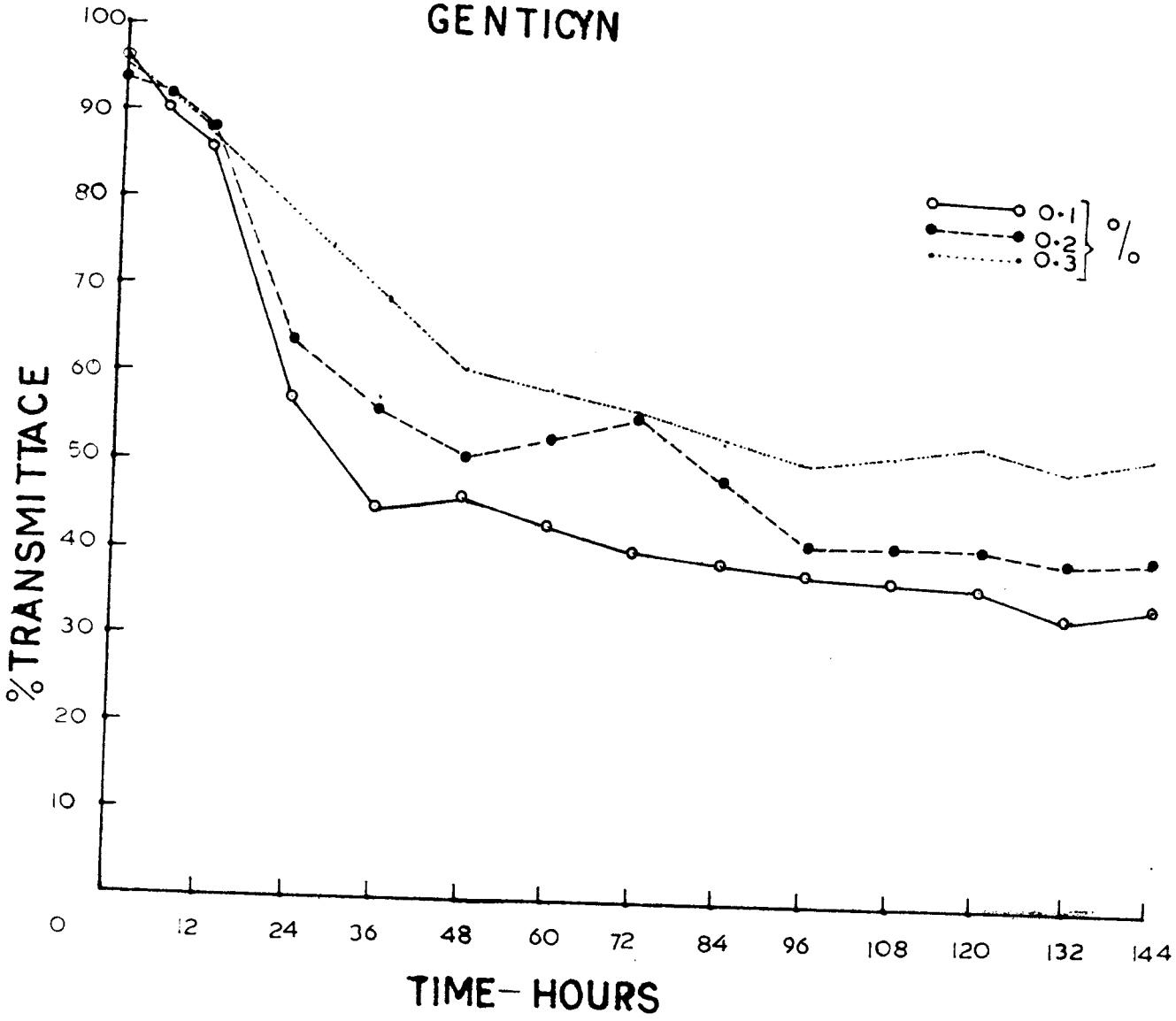

GENTICYN
100
90
80
70
60
50
40
30
20
10
0
% TRANSMITTACE
0·1
0·2
0·3
%
12
24
36
48
60
72
84
96
108
120
132
144
TIME– HOURS

FIG. 16

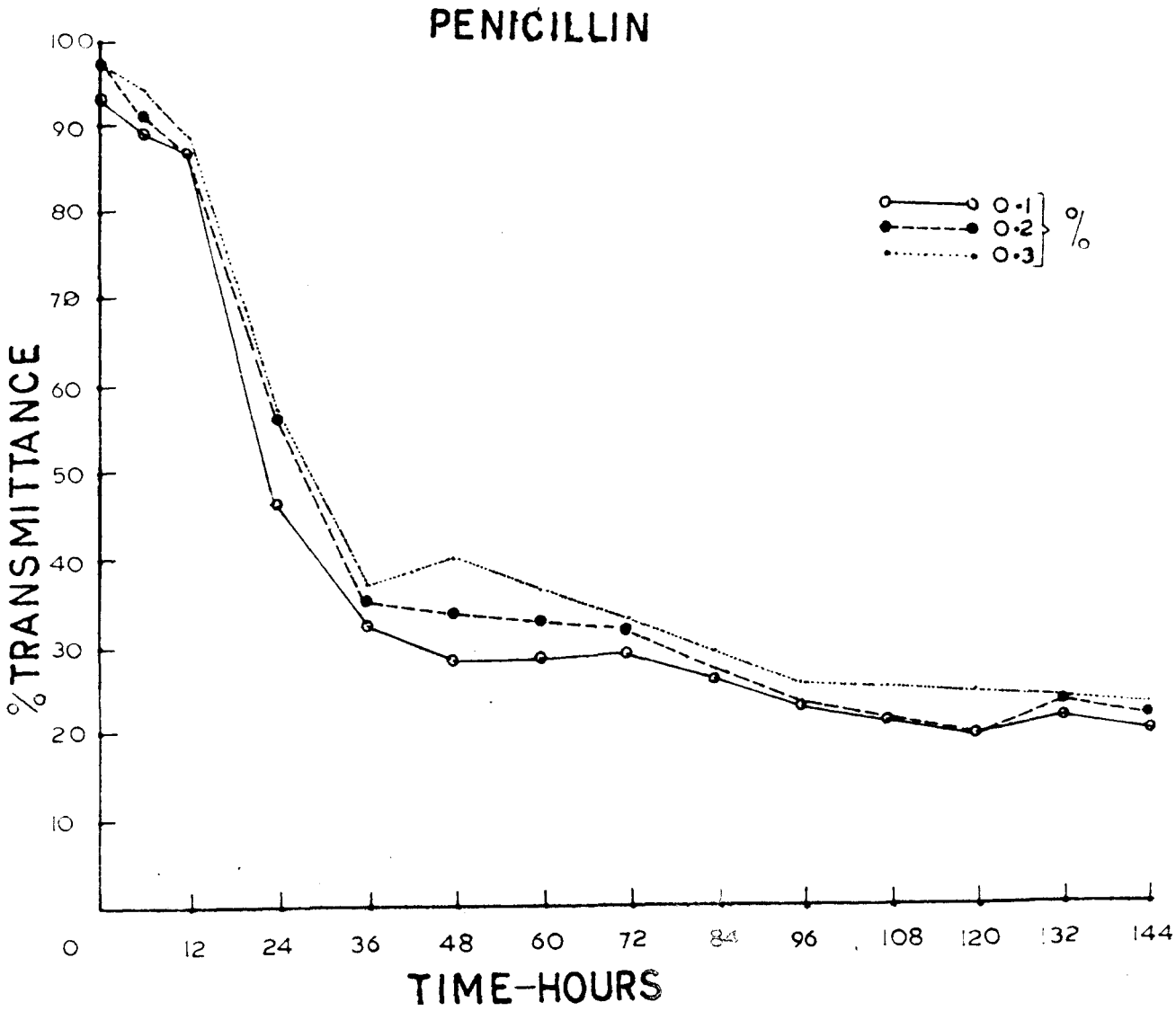

PENICILLIN
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
% TRANSMITTANCE
TIME-HOURS
0·1
0·2
0·3
%

FIG. 17

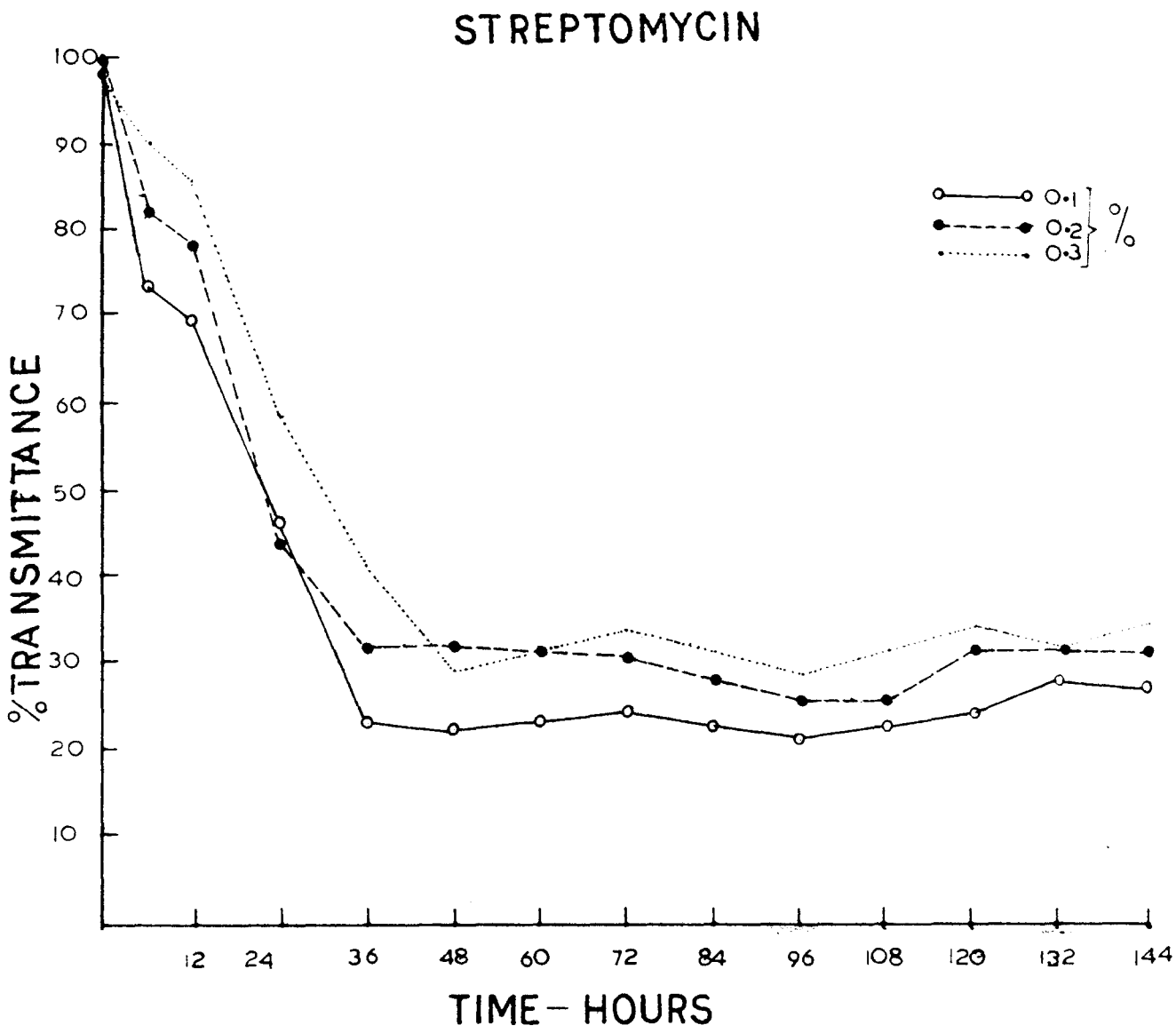

STREPTOMYCIN
100
90
80
70
60
50
40
30
20
10
% TRANSMITTANCE
0·1
0·2
0·3
%
12
24
36
48
60
72
84
96
108
120
132
144
TIME – HOURS

FIG. 18

Figure 19-24. Effect of various concentrations of insecticides on the growthof Klebsiella sp. (in terms of transmittance percentage) From 0.0 hr to 144 hrs.

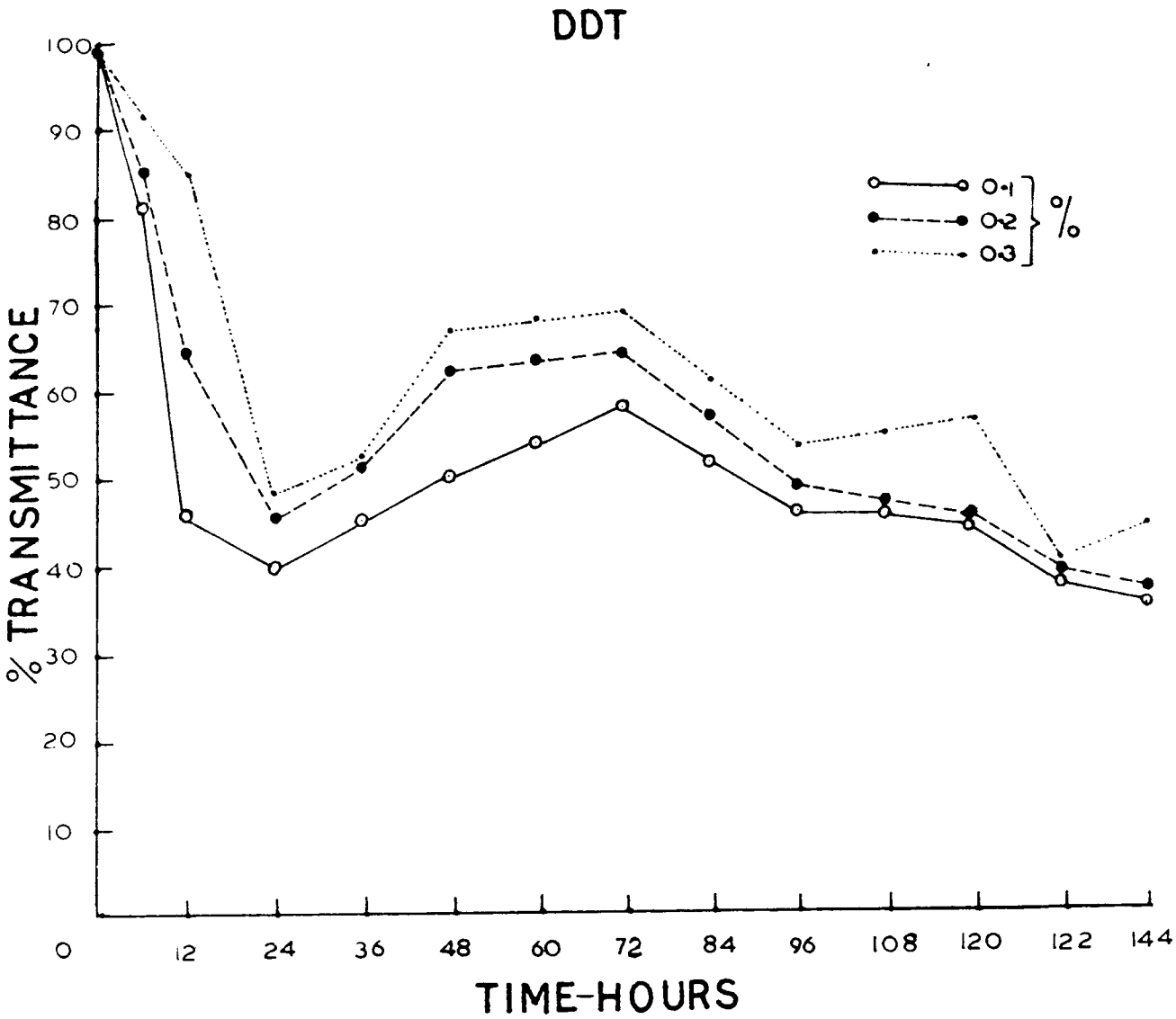

DDT
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
122
144
TIME-HOURS
0·1
0·2
0·3
%

FIG. 19

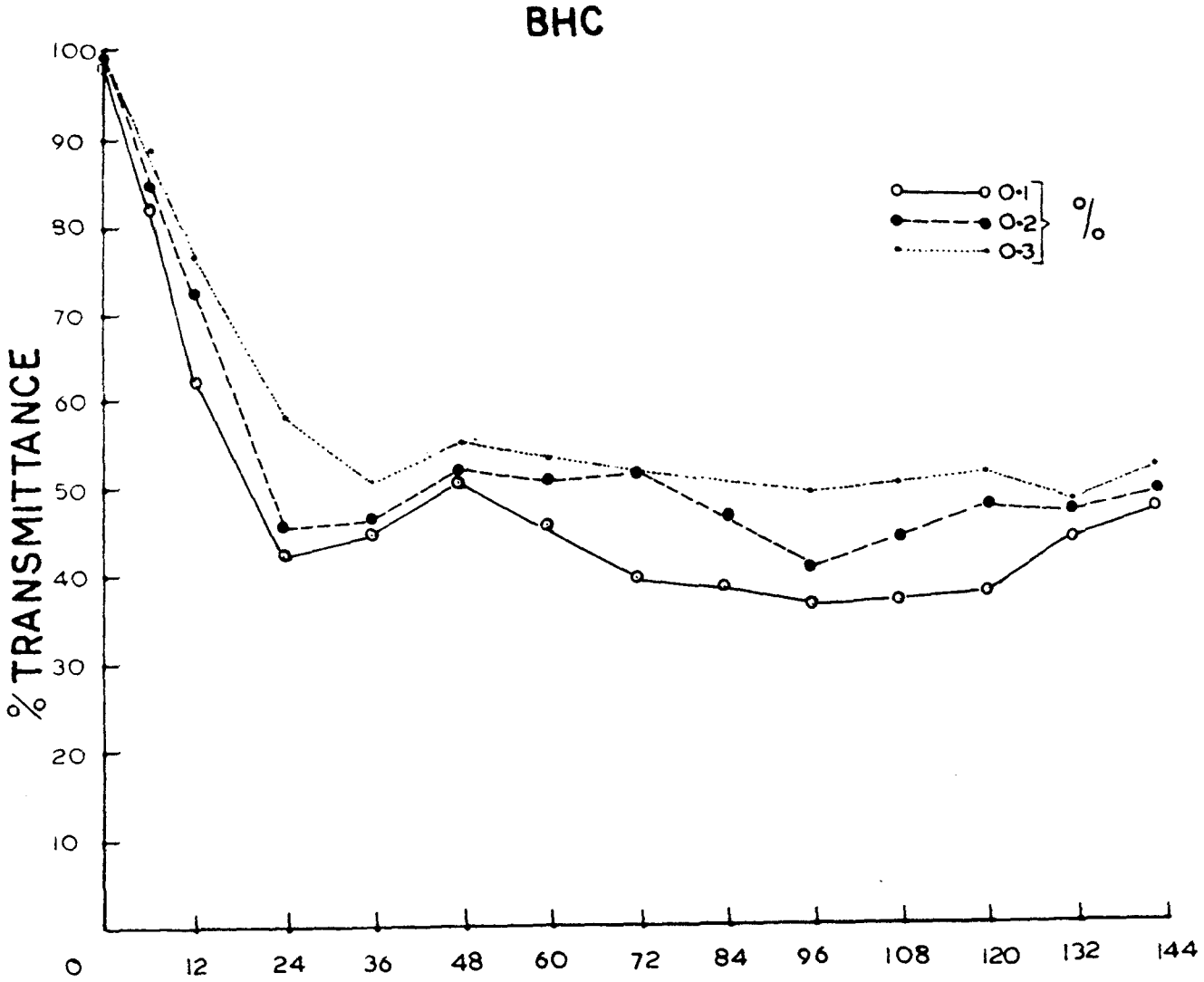
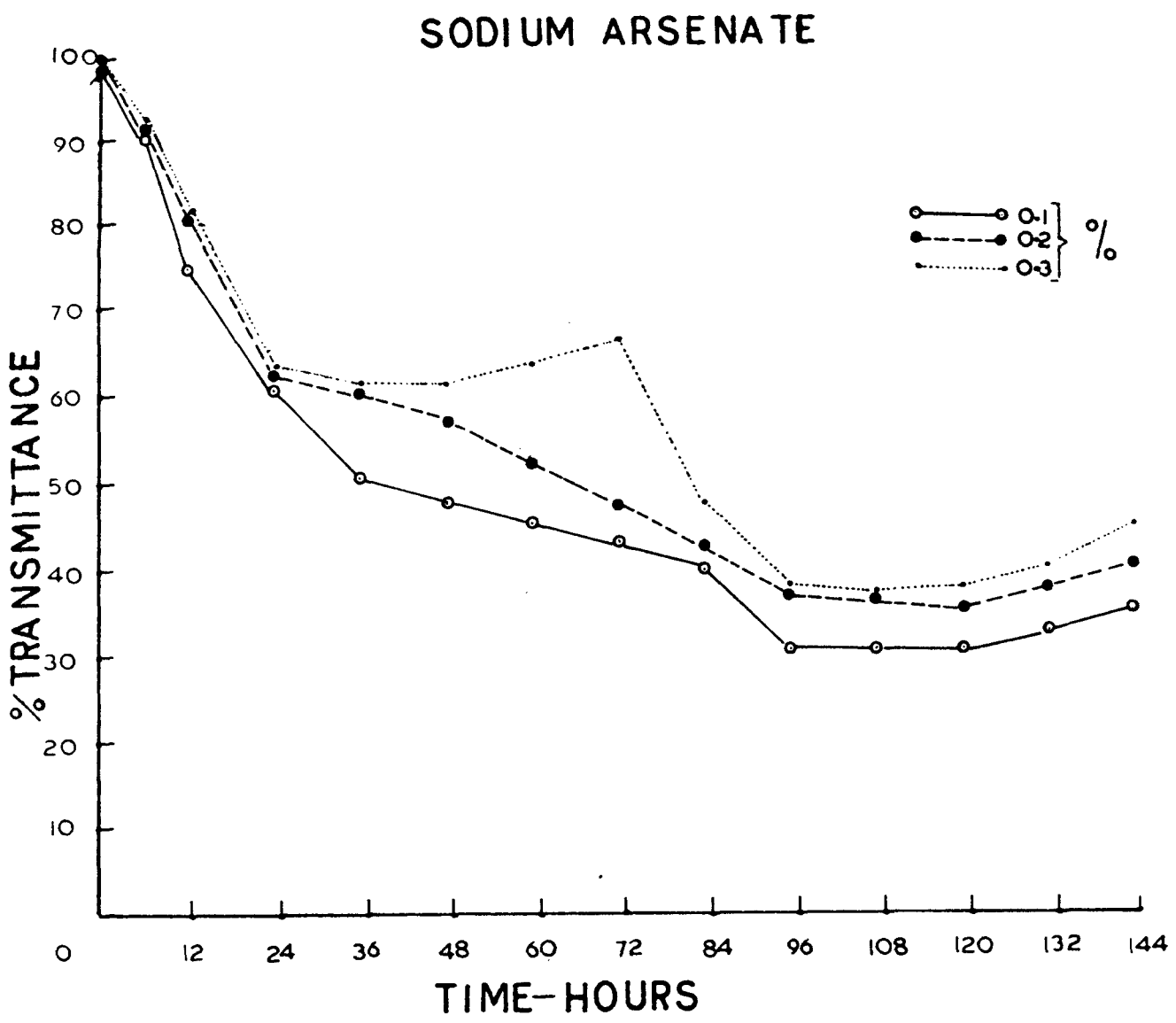

BHC
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
% TRANSMITTANCE
TIME-HOURS
0·1
0·2
0·3
%

FIG. 20


SODIUM ARSENATE
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME-HOURS
0·1
0·2
0·3
%

FIG·21

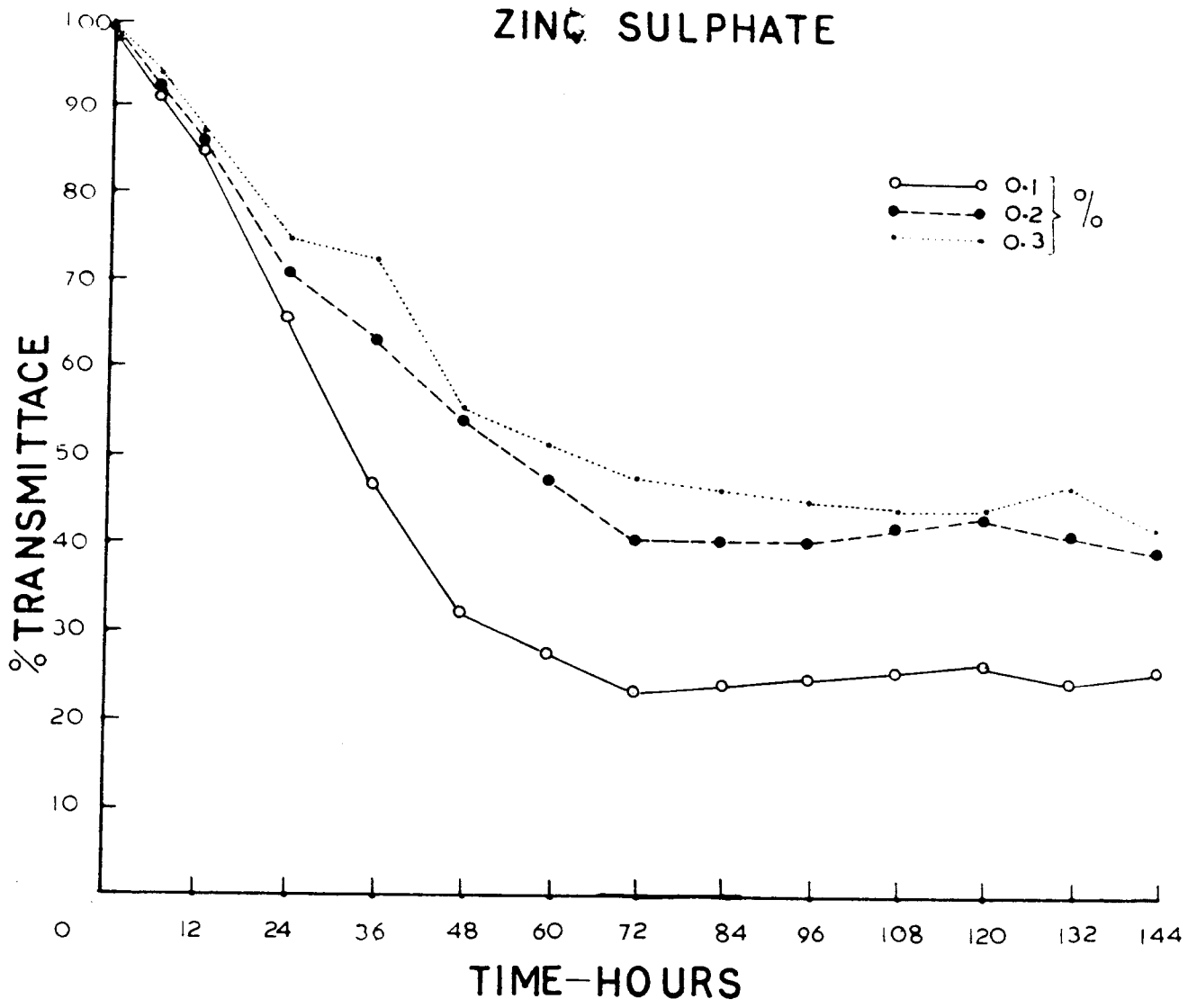

ZINC SULPHATE
% TRANSMITTACE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME–HOURS
0·1
0·2
0·3
%

FIG. 22

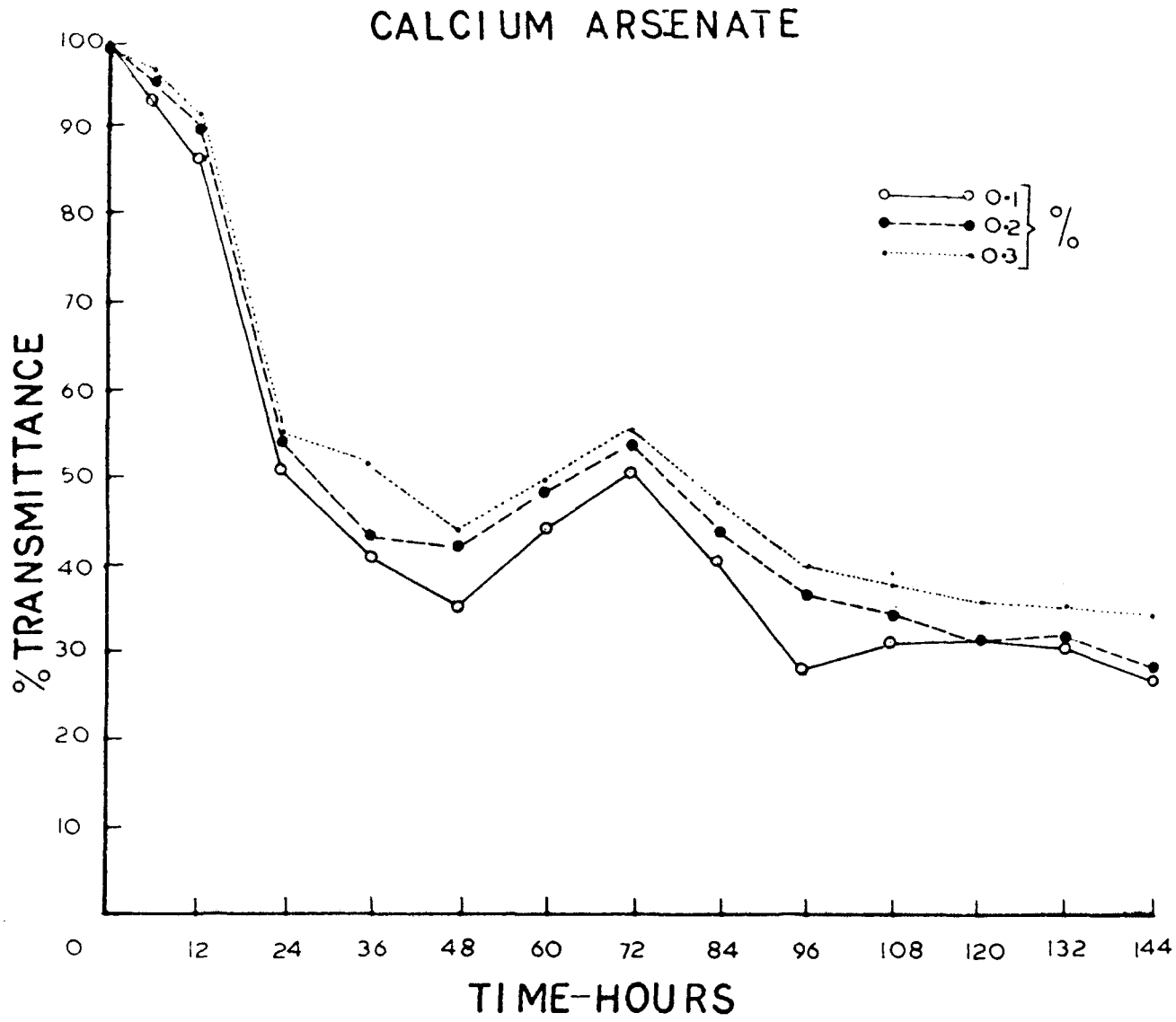

CALCIUM ARSENATE
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
TIME-HOURS
0·1
0·2
0·3
%

FIG. 23

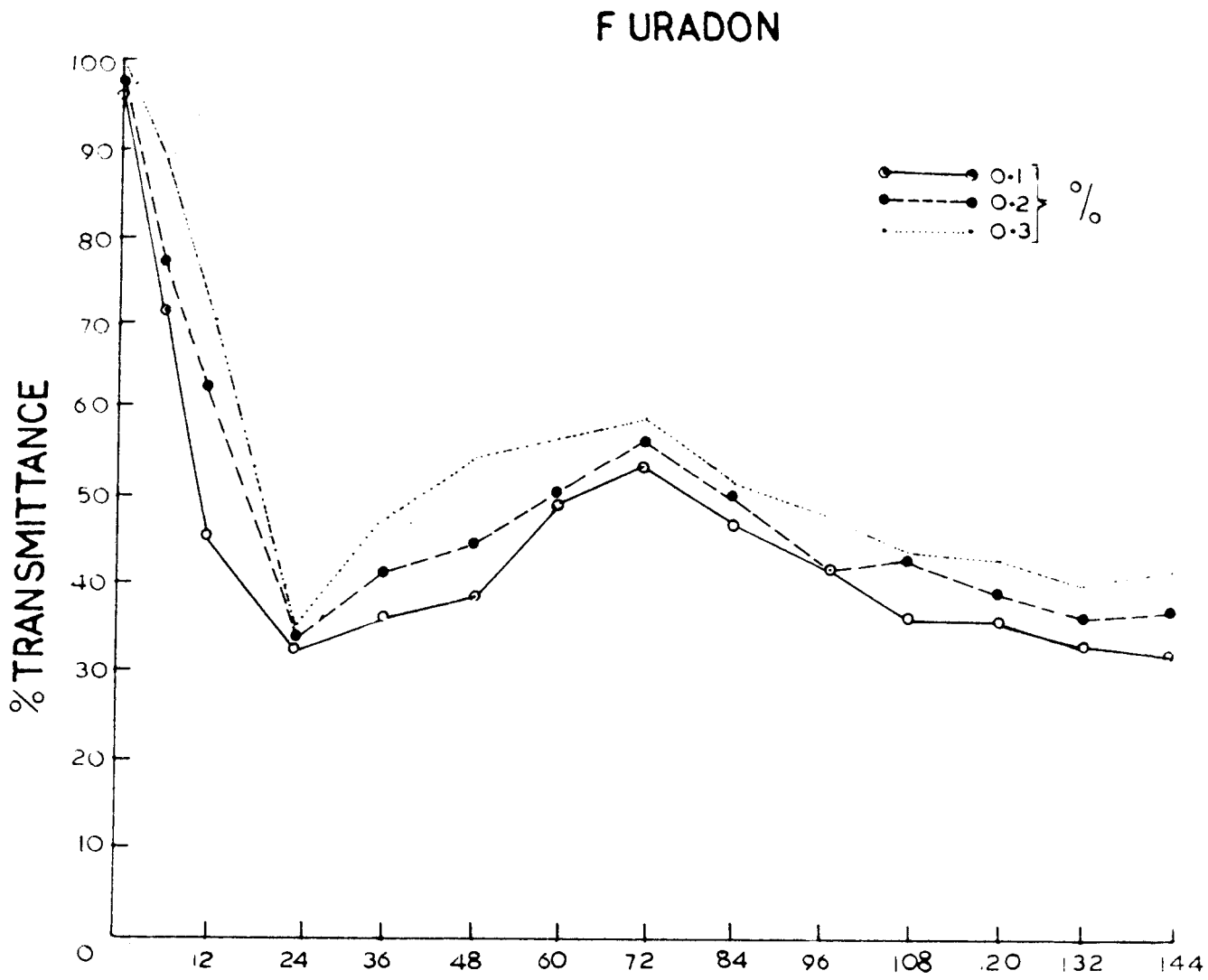

FURADON
% TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
132
144
0·1
0·2
0·3
%
TIME – HOURS

FIG. 24

ACKNOWLEDGEMENT

A C K N O W L E D G E M E N T

I failed to find suitable words to express my gratitute of my indebtness to Dr. Abeer H. Khan for his guidance and taking pain in going critically through the manuscript.

I am thankful to Prof. S. Mashhood Alam, Head, Department of Zoology for providing necessary facilities.

Thank also due to Dr. M.K.S. Ghauri, Taxonomist at Common wealth Institute of Entomology, London, for Identifying the specimen.

Financial assistance given by C.S.I.R. is also duely acknowledged.

At last but not least I am thankful to my colleague Miss Sadequa bano and numerous friends for their encouragement and help.

(MUNNEY MIYAN)

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